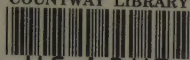


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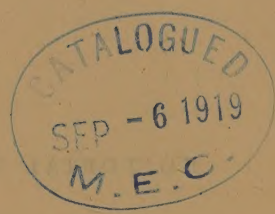
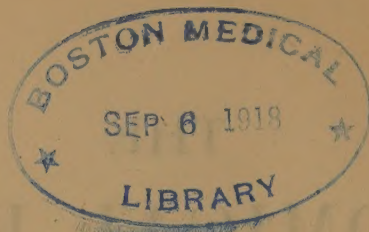
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Resumido por el autor, C. W. M. Poynter.

Algunas observaciones sobre la cicatrización de las heridas en el embrión joven.

El proceso de la cicatrización de las heridas ha sido estudiado por el autor en embriones de gallina muy jóvenes, observados en gota pendiente, y también en cortes. Las heridas producidas en el blastodermo extra-embrionario se cicatrizan a consecuencia de la actividad de las tres hojas blastodérmicas. En el embrión este proceso está limitado casi exclusivamente al ectodermo. En la cicatrización pueden reconocerse cuatro estados: 1, ajustamiento de las células; 2, desdiferenciación; 3, emigración celular; 4, rediferenciación. La desdiferenciación tiene lugar muy temprano en el proceso, tomando las células el mismo carácter, de tal modo que presentan el mismo aspecto y reacciones bajo la acción de los colorantes. Las células desdiferenciadas se vuelven a diferenciar de nuevo en células con el mismo tipo que las características de la hoja blastodérmica que las originó. La emigración de las células es de un carácter amiboide y la proliferación celular no toma parte en el proceso de la cicatrización.

Translated by Dr. José Nonidez
Columbia University



SOME OBSERVATIONS ON WOUND HEALING IN THE EARLY EMBRYO

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TWELVE FIGURES

While engaged in the study of degeneration of cells in the early chick embryo, it became necessary to observe the reaction of cells under the stimulus of an injury. The present paper is concerned with the reaction of tissues to injury and the suggested process involved in wound healing. The use of young embryonal material, on account of its elemental structure, permits us to study the healing process under somewhat simplified conditions and by direct observation.

Chick embryos ranging from ten to twenty-nine somites were selected for the experiments. Eggs were incubated for from eighteen to forty-eight hours, then opened under aseptic precautions and the embryos injured with a fine knife or hot needle; some were then placed on the hanging drop for immediate observation while others were sealed and returned to the incubator for various periods up to one hundred and twenty hours. The operations consisted in incisions of various parts of the blastoderm, removal of sections of tissue and cauterization of the embryo at different points.

For periods of short duration up to four hours the hanging-drop method was employed and direct observations made; all of this material was also sectioned for additional study. This method has been so frequently described that it is not necessary to review it in detail. It was originated, I believe, by Harrison and has been developed by a large group of workers, notable among whom are McWhorter and Whipple ('12), who first applied it to the study of the entire embryo.

For this study I have used the technique suggested by Margaret R. Lewis ('11), growing the embryo in Locke's solution without dextrose. An electrically heated stage incubator was employed in which it was possible to keep the chick alive for seven hours. I found it most satisfactory to transfer the embryo from the egg to warm Locke's solution for removing the vitelline membrane and washing away the excess yolk before placing on the hanging drop. In some cases the embryo was operated on while in the solution, but it was generally more satisfactory to first place it on the cover-slip; this facilitated handling, permitted operation under high-power observation when desired and allowed adjustment in the moist chamber with the least disturbance of the embryo. It is desirable to use aseptic precautions throughout the manipulations and very necessary to keep the embryo very near incubator temperature. For the observation of the growth process I used a monobjective binocular, for it permits the use of high magnifications, produces a certain amount of stereoscopic effect and, what is more important to me, permits several hours' continuous observation without producing excessive fatigue of the eyes. When any observed process reached a desired point the chick on the cover slip was fixed in osmic acid and so carried into paraffin. When in 70 per cent alcohol, slight care to see that the fluid circulates freely between the embryo and the glass will insure the easy removal of the embryo from the cover-glass in the embedding process. The usual methods of sectioning and staining were employed and merit no comment.

OBSERVATIONS ON THE LIVE CHICK

Some of the changes which may be observed in the live embryo appear so simple as to be hardly worthy of record, and it is only when one remembers the large amount of material and the hours of patient work necessary to gain the same information from slides that the value of this method is realized. It must also be remembered that this method gives added value to the stained sections, for a process can be watched till it reaches the desired stage or one difficult of interpretation and then fixed.

After injury changes take place very rapidly, so it is desirable to place the hanging-drop preparation under the microscope as soon as possible. If a wound is examined a minute or two after it has been made the edges appear ragged and broken. The border is made up of fragments of cells and free cells. Within five minutes the borders of the wound have become smooth in outline due to the readjustment of cells. The broken cells are carried away and the free cells move about till all irregularities are overcome. The cells from one germ layer do not become incorporated in the other germ layers, but these readjustments take place independently in each layer. In observing the behavior of the cells during this period one is reminded of the repulsion and attraction which electric poles have for a pith ball. There is a noticeable current in the fluid of the drop directed away from the wound; this is probably an out-pouring of the fluids of the tissues and is undoubtedly one of the factors involved in the movement of the debris away from the wound margin. In the case of certain detached cells there is an attraction sufficiently strong to overcome the movement of fluid away from the wound and cause them to move back again in contact with the undisturbed cells and become incorporated in the growing margin. This behavior of the unattached cells suggests the experiments of Driesch ('96) who, by shaking, displaced the primary mesenchymal cells of *Echirus*. In the course of a few hours these scattered cells rearranged themselves so that development proceeded in a normal way. Both are examples of what Roux ('96) called 'cytotaxis' and which he suggested was due to local differences in the superficial tension of the cells. I have not seen injured cells, i.e., cells which have had part of their cytoplasm cut away, incorporated in the growing wound margin, but think farther observations should be made on this point.

Observations on the extra embryonal blastoderm show that all three germ layers take part in the healing process. The ectoderm and the entoderm are somewhat more active than the mesoderm. Within thirty minutes after the operation the borders of the wound are noticeably thicker than the region farther back and the rent is narrower than in the beginning

(fig. 1). The piling up of the cells at the edge of the wound is a constant feature of all wounds observed in the extra-embryonal blastoderm. It would seem to be an expression of the difference between the gradient resulting from wound stimulus and the force of surface tension of the germ-layer mass.

At this time all of the cells of the wound margin are round and more transparent than those of the undisturbed blastoderm. They may be said to have taken on an indifferent character for the individual cells in their morphology furnish no indication of the germ layer from which they have been derived. I must make an exception to this in the case of the cells of the entoderm of the area opaca which may still be identified by the yolk granules present in the cells. The cells of the different germ layers are alike only in appearance. Dedifferentiation has occurred to the extent that the characteristics of the parent germ layer are lost and to this extent the cells are in an indifferent state. They are, however, only relatively indifferent for, at least as long as normal stimuli operate, each cell will remain in the germ-layer group of common origin and later differentiates, or redifferentiates, into the germ-layer type from which it sprung. At no time during the healing process are the cells distributed evenly over the wound, but those derived from each layer remain together to build up, or advance, that layer. I have never observed any cell from one layer become incorporated with those of another layer, even when the relations seemed most favorable for such an adjustment. For example, when examining a border in which the ectoderm was slightly behind the other layers none of the cells pushed in to fill the gap. This is illustrated in figure 2.

The process of healing, or building up of the wound margin, is difficult to interpret, notwithstanding that the process takes place immediately under the eyes and can be readily observed. At first, as cited above, there is an adjustment of cells on the edge of the wound so that the surface becomes regular and in the case of syncytium an adjustment of cytoplasm about the border nuclei with the formation of recognizable cell membranes (fig. 1). The cells near the border seem to become loosened

from each other and approach spherical shape. The cytoplasm increases, becomes less granular and the nuclei and nuclear membrane almost indistinguishable. There is a forward movement of the entire tissue mass and frequently at some distance from the wound border individual cells may be seen moving toward the border more rapidly, edging their way among the other cells till they reach the margin where they take on the characters of the border cells. The healing process is quite rapid; a fissure 2 mm. wide had entirely closed in two hours and twenty minutes, leaving only a slightly more transparent area to indicate the line of juncture. As soon as the two wound borders come in contact the cells of the thickened borders begin a readjustment which soon leaves the cell layers of the same uniform thickness they were before the wound was produced. The cells of the borders gradually resume the typical appearance of those in the undisturbed layers and within four hours no visible evidence of a wound remains. The activity of the border distal to the embryo seems to be as great as that next to the embryo. An observation of the wound margin for some time suggests that growth activity is not constant for the whole. This produces a slightly wavy appearance; for example, an area was noticed which advanced for a time more rapidly than that on either side of it; then a period of decreased activity ensued of such duration that the area became the most retarded in the region and appeared as a depression; later growth activity was resumed. I can only suggest that possibly the products of cell metabolism, which on account of the great activity are not eliminated, act as an inhibitor to cell movement.

Injuries of the embryo present a slightly different picture. If the wound borders are close together, in contact, there is a shifting of the cells of the wound margin and the appearance of the clear cells already described which come in contact with those of the opposite side and so produce union per primam intentionem. All of the germ layers seem to take part in this process and union is brought about through cell dedifferentiation and adjustment.

When tissue is cut away, as in the removal of a lateral portion of the embryo, there is a readjustment of cells similar to that described for the extra-embryonal blastoderm, then the process becomes very much slower. During a four-hour period of observation the ectodermal and entodermal borders could be seen to advance slowly over the exposed mesenchyme by the process noted above, with the exception that the ectoderm was much more active than the entoderm and the cells of the border did not tend to pile up. The only response of the mesenchyme to wound stimulus seemed to be a shifting of cytoplasm so that a continuous cytoplasmic border could be seen. This border was like a cell membrane, but, except in the case of the mesothelium, was never observed to produce a distinct layer of surface cells.

The behavior of the spinal cord was observed in various regions, but may be best studied in transverse sections. As in the other tissues, immediate proximity of the cut surfaces seems to stimulate cell activity, and primary union is observed to take place in two to three hours. In the case of an exposed surface reaction is very slow. Adjustment of cells closes the end of the canal and covers the surface with closely arranged cells. This surface layer is derived by the shifting of cells. It seems that certain cells which are exposed by the cut do not take part in this general movement, but are finally covered by other cells which come to occupy their place in the superficial layer. Careful observation has failed to discover any distinguishable difference between cells which are active in forming the surface layer and those which are covered by it.

From the most careful observations I was able to make I did not discover an instance of indirect cell division. The healing process seems to be accomplished through a general movement of cells and cell layers and changes in the cells which, with modification, may be called dedifferentiation; only at a later period can we consider normal cell multiplication as a factor in wound repair.

STUDY OF SECTIONS

Figure 1 is taken from experiment 170. The age of this embryo at the time of operation was about forty hours. The operation consisted in making a long cut in the outer border of the area pellucida, about opposite the heart and to the right of the embryo. The wound margins separated widely, due to the pressure of the yolk. After incubating for two hours the blastoderm was fixed for examination.

The edges of the wound show the piling up of dedifferentiated cells at the border of each germ layer as already described in the preceding section. These cells are large clear cells with distinct cell membrane, they take the stain lightly and those of one cell layer are indistinguishable from those of the other layers. All examples of the early periods of the healing process show that the activity of the three germ layers of the extra-embryonic blastoderm is nearly the same. There are no evidences in any of these sections of indirect or direct cell division.

Figure 3 is taken from experiment 172, in which the wound was a tear made with a fine needle with the object of removing only the ectoderm layer. The experiment was fairly satisfactory, for the major portion of the wound consisted of an area of from $\frac{1}{2}$ to 2 mm. broad denuded of ectoderm; at one end of the wound the needle entirely pierced the membranes, which was an advantage, for it permitted the study of different degrees of injury for comparison. The figure shows an area in which there has been a union of the two torn margins of ectoderm. All of the wound margin is made up of dedifferentiated cells, and when, as in the figure, the borders have fused there is as yet no evidence of redifferentiation. In this experiment the healing process was allowed to proceed for seven hours.

A study of these sections shows that the wound stimulus is not transmitted from an injured to an uninjured cell layer. When, as in the figure, the ectoderm is destroyed there is a reaction of the border cells of this layer but none of the mesoderm although it is exposed. Wound stimulus is apparently directly related to the injury or separation of cells in any cell

layer, the different layers behaving for the time being as separate individuals. It seems that the ectoderm is uninfluenced in the speed of its reaction by the presence of the other layers. A wound, for example, 1 mm. broad involving the ectoderm alone closes in the same time as one involving all three layers, other conditions being the same.

Experiment 173 was an operation similar to those described above, on an embryo forty-eight hours old with five hours as the duration of the healing process. This wound was almost 2 mm. broad with rough edges and included all three germ layers. When examined at fixation, the wound was found to be entirely closed. The wound area was very easily made out, for the juncture of the two margins was marked by a thickening due to the large number of indifferent cells present. The division between the ectoderm and mesoderm is not distinguishable nor is it possible to discover any line of juncture of the wound borders. All of these dedifferentiated cells appear so much alike that there are no physical characters to suggest from which germ layer any of them have sprung. At this stage, while there is no indication of a wound border it has been found that the wound can be easily reopened. Repeatedly in experiments showing perfect union at fixation, the most careful handling has not prevented a ruinous tear before the material could be safely embedded.

Experiment 201 is illustrated in figure 5. A blastoderm about twenty-four hours old had a large hole torn in the extra-embryonic blastoderm. The egg was resealed and allowed to incubate for twenty-four hours. When the blastoderm was removed from the yolk a large hole still remained, but the edges of the opening appeared smooth and healed. The stained sections show that the wound margins are filled with indifferent cells and that through these the germ layers are fused. Except in primary union, I have not found the germ layers of the border united in this way earlier than eighteen hours after the injury was inflicted.

Figure 6 is taken from experiment 11 in which the conditions were the same as in experiment 201 except that the healing proc-

ess was allowed to proceed for twenty-eight hours. In this experiment the germ layers are still separated and the picture very much resembles figure 1, only there are more redifferentiated cells at the border of each layer. It seems that the three germ layers remain separate at the wound margin, as in figure 6, till such time as the freedom from embryonal dominance produced by wound stimulus is overcome, then the indifferent cells fuse into one mass and redifferentiation begins. The mass of dedifferentiated cells seldom exceeds in size that seen in the figure. In wound margins of this type, which are twenty-four hours old or older, the outer cell layer of the indifferent mass frequently shows degenerative changes or even distintegration. These changes are probably due to lack of nutrition.

Experiment 28, shown in figure 7, was an operation similar to that of experiment 201, in a blastoderm of twenty-hours' incubation. The healing process was allowed to proceed for fifty-four hours. In this, as in all experiments when the healing process has been allowed to continue for forty-eight hours or more, the fusion of the border layers is complete and redifferentiation has occurred. The ectoderm joins the entoderm so that the line of juncture is hardly discernible and the mesoderm fuses with mesoderm to form a single continuous plate.

Wounds of the amnion were observed to behave in the same way as those I have just outlined, so it is unnecessary to repeat the observations made on this membrane. Barfurth, '02, and Lillie, '03, have both observed the closure of wounds of the amnion.

Experiment 212 was made on an embryo about thirty-four hours old. A cut was made parallel to the long axis of the embryo and a short distance lateral to the spinal cord, completely dividing all of the tissues. The wound edges were separated widely, the egg resealed and allowed to incubate for five hours. The sections show the difference in reaction between the embryo and the extra-embryonic blastoderm. The denuded mesenchyme shows a definite border where the readjustment already described had occurred, but there has been no active dedifferentiation of cells. The advancing border of ectoderm

shows dedifferentiated cells at its edge, but there is no tendency for these to pile up in the way observed above. The entoderm shows much less reaction to the wound stimulus than the ectoderm. At later stages when the ectoderm has almost covered the exposed mesenchyme of the somatopleure there is a shifting of cells of the mesenchyme, so that the coelom becomes closed through the fusion of the two mesodermal plates. The process seems to be brought about, at least in part, by shifting and fusion of dedifferentiated mesothelial cells. Figure 8 is taken from this experiment.

Figure 9 is taken from experiment 54 in which conditions were the same as in experiment 212 except that healing was allowed to proceed for twenty-four hours. This shows the two plates fused, covered by ectodermal epithelium and the healing process complete except for the redifferentiation of mesodermal cells where the two mesenchymal plates joined.

If a large area has been denuded of its epithelium it will take more than twenty-four hours for it to become covered. In all wounds of the embryo the greater activity of the ectoderm as compared with the entoderm is very noticeable. The tendency of dedifferentiated cells to pile up along the advancing border of either ectoderm or entoderm is very much less than in the extra-embryonic blastoderm. After about twenty-four hours mitotic figures can occasionally be seen in the cells of the wound margin, but never in sufficient numbers to warrant the conclusion that cell division is more active here than in regions more remote, nor that cell division, even at this time, plays a major rôle in the healing process.

A study of wounds of the spinal cord shows that there is no apparent change after the first readjustment of cells observed in the section above. After a longer or a shorter time the ectodermal epithelium covers the exposed cord tissue in the same manner as that just described for the mesenchyme. Figure 10 is taken from an oblique section cutting through a transected cord in which the healing process had proceeded for twenty-four hours. The section shows the advancing border of the epithelium and the reaction of the cord cells on the wound margin.

Figure 11 taken from another experiment on the cord is shown to illustrate the closure of the neural canal. I have already spoken of the early closure of the canal by a shifting of cells. This is followed by a collapse of the canal for a distance of from 0.3 to 1 mm. from the transection. The two lateral walls of the cord come into contact as if through lateral compression and the canal is entirely obliterated. There is, however, no true fusion of the cells of the two lateral halves of the cord and a distinct line of separation can always be made out.

All of the examples I have used in this study have been from incised or torn wounds. The experiments in which the hot needle was used were not entirely satisfactory. It was necessary to work very rapidly with the needle or it would become too cool to cauterize properly. This made it impossible to do careful operations or exactly limit the amount of damage to the tissues. If the needle was too hot it tended to stick to the tissues and in freeing it much unintentional damage was inflicted. In the experiments studied there was the same type of reaction to wound stimulus as when a simple incision was made, but the process was much more difficult to interpret because of the injured cells and necrotic tissue present. Figure 12, taken from experiment 62, shows the effect of forty-eight hours' healing of a wound of the somatopleure produced with a hot needle.

The experiments cover observations on embryos as young as the ten-somite stage and as old as the twenty-nine-somite stage. No differences in the degree or type of reaction have been noted for the different ages. Observations were made up to 120 hours after the injuries were inflicted. This is a longer time than is necessary for processes covered in this paper, for redifferentiation is complete in most cases by the end of sixty hours. The later observations suggest that the chick may survive very serious mutilation and go on developing in a normal way but that it is incapable of regeneration in the sense that the term has been used for the simpler animals. Simple wound healing seems to be the only reaction to wound stimulus of which warm-blooded animals are capable and in this respect the embryo, at least the chick embryo, reacts as does the adult.

DISCUSSION

The reaction, common to all animals, by which wound surfaces become closed through a process called healing has long been recognized. Within the last two hundred years observations and experiments on the possibilities of regeneration have broadened our knowledge of this important reaction and suggested problems of the greatest biological interest. The earlier studies concerned themselves with the behavior of organs and tissues of adult vertebrates. More recently the ability of different animals to regenerate lost parts or to regenerate an entire individual from a fragment has received the major attention of biologists. The general questions concerning the extent of regeneration, its histogenesis and the conditions effecting it furnish a field for research of the most fundamental importance.

Wound healing seems to be one step or phase of the general process of regeneration. In the lower animals the closure of the wound is followed by continued local growth leading to a more or less complete restoration of lost parts. The higher we go in the animal scale the greater the complexity of organization and the less the power of a part to reproduce the whole. Among the higher vertebrates almost all that remains of the process of regeneration is the reaction of wound repair. Wound healing, particularly in man, has been very extensively studied. There are, however, many unsolved problems still confronting us. A comparative study of the regenerative process in embryos and adults of the higher vertebrates has received very little attention. The reaction of the embryo to wound stimulus offers an opportunity to study the process under somewhat simplified conditions and not only adds to our knowledge of the problem of regeneration, but throws light on the general growth question as well.

Fraisse ('85) studied the regeneration of epidermis on the tail of amphibian larvae and gave an historical account of epithelial regeneration for vertebrates. He concluded that, in adults, the epithelium regenerated from epithelium by cell proliferation. This was contrary to the generally accepted theory that epi-

thelium might arise as the result of a metamorphosis of leucocytes and contrary to his own observations on larvae, or rather in spite of the fact that he saw no evidence of cell division nor new cell formation.

Barfurth ('94) studied regeneration of the germ layers of amphibian larvae. He noted that the time of closure of the wound was directly dependent on its size and that the ectoderm reacted more rapidly than the entoderm, due, he believed, to its greater elasticity and the firmer manner in which the cells are held together. He made a sharp distinction in the healing time between a cut and a tear, finding that a clean cut healed more rapidly. In the chick there is very little difference in the reaction time of the different germ layers of the extra-embryonic blastoderm, and except in the time of adjustment, which is negligible, it seems to make no difference whether a wound is incised or torn. Barfurth noticed the piling up of cells, "consisting solely of cells torn from the layer," which he called, after Roux, *Extraovata*. He said, "The *extraovata* after reaching a certain size comes under dominance of the embryo. If it passes beyond the dominance of the embryo it will develop into a separate embryo." It is probable that the *extraovata* is of the same character as the mass of indifferent cells I have noted on the border of the wound in the chick. In the chick, however, the mass does not reach a size nor behave in a way to suggest an *extraovata*. The higher organization of the chick and the consequently greater dominance of the embryo probably accounts for the difference between the two forms.

Born (96) also studied amphibian larvae and concerned himself with the way in which the cicatrix was covered by the epithelium. He observed (p. 579): "On account of the time in which the epithelial covering is completed, mitotic division of cells is not to be thought of. It appears to me, that the epithelium as a whole is concentrically shifted (*vergeschoben*) over the wound surface,—for the picture does not suggest an active wandering out of the individual cells." As already pointed out, I am in entire agreement with the conclusions that there is a general shifting of the epithelial layer and absence of cell divi-

sion, but in the chick the border of the wound suggests the locus of greatest cell reaction and I see no evidence for the conclusion (p. 572) that the movement of the whole layer is due to the vital effort of the individual cells to flatten themselves over the greatest possible surface. It is impossible to observe the loosening up of cells near the border and the behavior of the cells at the extreme margin without being impressed with the fact that the advance is an active, not a passive one. I must conclude, as Rand ('05) has for the earthworm, that "We are compelled to look in the individual cell itself for the immediate source of activity."

Oppel ('13) and Osowski ('14) studied the behavior of the epithelium on explants of frog larvae and concluded that the movements of the epithelium are responsible for the covering of the wound, not through some pressure behind, but as a direct result of the activity of the cells themselves. Osowski speaks of the action as due to 'Massenbewegung.' He observed no pseudopodia, consequently does not look on the movement as amoeboid in character.

Holmes ('14) repeated the work of Osowski but reached a different conclusion concerning the way in which the cells moved. He decided that "The extension of epidermis in both larval and adult forms is due to the amoeboid activity of the hyaline protoplasm along the margin of the extending mass." I was unable to observe pseudopodia on any of the advancing cells, but this is valueless as negative evidence, for Holmes pointed out that, "The pseudopodia of epithelial cells of amphibian larvae are so short and so fine that it would scarcely be possible to detect them when they are extended over other parts." To morphologists who find satisfaction in tangible structure, the discovery of pseudopodia will offer additional proof of individual cell activity.

Harrison ('14) has shown that cells growing in vitro possess stereotropism and suggests that this may explain in a measure cell movement on wounds. The phenomena of stereotropism and cytotaxis, already alluded to, suggest that the movement of epithelial cells is in response to a direct stimulus of chemico-

physical nature. We have already seen that the behavior of the ectoderm of the extra-embryonic blastoderm is different from that on the embryo. If the behavior were the same we would not have a gap in the blastoderm bridged, but the ectoderm would immediately advance over the mesoderm to unite with the entoderm and a healed border would result; a reaction which does not take place till several hours have elapsed, and then only in extensive wounds. May it not be that in tissues remote from the embryo the wound stimulus, for a time, frees the cells of the border from the natural gradients and makes them behave as independent individuals; later as the stimulus is exhausted the normal gradients are reestablished and the ectoderm behaves as it does on the embryo?

Lillie ('03) studied the powers of regeneration of various organs in the chick. He was interested primarily in correlative differentiation, but recognized the existence of wound repair in these embryos. Shorey ('09) also studied regeneration in the chick. Both observers concluded that, beyond wound repair, no regeneration is to be expected for the chick.

I have referred to the changes which take place in cells of the wound margin by which they lose their distinguishing germ-layer characters. These cells may be said to have dedifferentiated or become indifferent, but they always take on again later, when normal conditions are reestablished, the type form they had before the wound stimulus was operative. So much has been written of dedifferentiation in recent years that it is not necessary to review the literature. In lower animals, as those in which a whole part or individual may regenerate from a fragment of tissue, the commonly held theory seems to be that the new part or individual is developed by the dedifferentiation of the old tissue cells and their redifferentiation into tissues of the new individual.

Minot ('08) presented a different view, he said "If the head or tail of a planarian is cut off the part lost is regenerated, not by growth of the old tissue, but perhaps wholly by multiplication and differentiation of 'formative' cells which migrate to the place where they are needed and produce the structure required."

Again he called these cells "of embryonal type, that is to say, cells of the young type." It would seem that there is much experimental evidence lacking to establish the theory of 'formative' cells, and while there are many connective-tissue cells which are difficult to classify, their behavior is not sufficiently understood to warrant the conclusion that they are 'young cells' capable of differentiation in any direction.

If we accept the theory that cells under certain conditions are capable of dedifferentiation and redifferentiation it may be qualified, for an examination of the experimental work in this field indicates that the potentialities of dedifferentiated cells are influenced by certain known factors. The cut end of a planarian may be made to grow either a head or a tail at the will of the experimenter; but as we advance to animals of more complex organization the extent of regeneration becomes less and less. Child ('15) has pointed out, "That the inhibition or retardation of new individuation by the dominant region of an individual occurs when the original gradient is sufficiently fixed in protoplasm." That would mean that the further we advance in the animal scale the greater the dominance of the 'original gradient,' hence the more limited the process of regeneration. If the original gradient limits the process of regeneration, it probably does so through determining the type of redifferentiation of the dedifferentiated cells and through limiting the extent of dedifferentiation. The indifferent cells which appear on the wound border of the chick, as the result of wound stimulus, are dominated by the original gradient or gradients to the extent that they redifferentiate only into the germ-layer type from which they sprung.

SUMMARY

Wounds of the chick blastoderm heal with great facility and the process can be watched for a number of hours in hanging-drop preparations.

In wounds of the extra-embryonic blastoderm all three germ layers take part in the healing process, but the ectoderm and the entoderm are somewhat more active than the mesoderm. As the result of wound stimulus the cells dedifferentiate and all take on the same general appearance and stain reaction. The dedifferentiated cells pile up along the border of the wound, but these indifferent cells of the three germ layers do not fuse and form a healed margin till many hours after the wound has been made. In the interval, before fusion, the masses of the three layers remain separate and advance by amoeboid movement of the individual cells till the opposite wound border is encountered when the two fuse. Later the dedifferentiated cells redifferentiate into cells of the type from which they sprung and wound healing is complete.

Wounds of the embryo heal by dedifferentiation of the ectodermal epithelium and the migration of these cells over the cicatrix. The process is through dedifferentiation, migration by amoeboid movement and redifferentiation into epithelium. There is no regeneration of the underlying parts and the entoderm takes very little part in the process. The directive stimulus which causes the migration of the epithelial cells is probably of a chemico-physical nature and the covering of the wound is effected without the occurrence of cell proliferation.

The wound stimulus is not transmitted from the injured to the uninjured tissue layers in the chick, and even dedifferentiated cells are so limited in their potentialities that regeneration, in the sense that it occurs in the lower animals, is not observed. It would seem that wound repair is a step or phase of the process of regeneration and that the embryonic dominance is so pronounced that it prevents the wound stimulus from carrying the process beyond this phase.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Section through the wound border of the area pellucida. From experiment 170 on chick forty hours old, wound healing of two hours' duration. Left margin shows the indifferent cells of the three cell layers. The tendency to pile up is most noticeable in the ectoderm. $\times 450$.

2 Section through the same wound shown in figure 1, but at a different level. When the three layers have relations which are favorable for fusion, they remain independent of each other. The clear indifferent cells are a constant feature of this stage.

3 Section through wound of experiment 172 in which healing process has proceeded for seven hours. The ectoderm only was wounded and the fissure has filled with indifferent cells so that the line of juncture cannot be discovered. The other cell layers have not reacted to the wound stimulus of the ectoderm.

4 Section through a wound of the extra-embryonic blastoderm which has just closed. Experiment 173 allowed to heal for five hours. Indifferent cells so packed together that it is difficult to distinguish those of the mesoderm. $\times 450$.

5 Section through wound of extra-embryonic blastoderm of experiment 201. Healing has progressed twenty-four hours. Somatopleure and splanchnopleure have fused and entoderm is continuous with ectoderm. At this stage the cell layers have lost the seeming repulsion which acted to keep them apart in earlier stage shown in figure 1.

6 Section through wound of same region shown in figure 5, experiment 11; wound repair time, twenty-eight hours. The two plates of mesoderm have fused, but the ectoderm and entoderm are still separate. Except for the migration of the wound border and a somewhat larger number of dedifferentiated cells, the figure is the same as figure 2, indicating a slowing up of the reaction produced by wound stimulus.

7 Section through healed wound from experiment 28. The process of repair has been allowed to continue for fifty-four hours. This is a later stage of the conditions shown in figure 5. The dedifferentiated cells have redifferentiated and it is impossible to discover the line of juncture of the two plates.

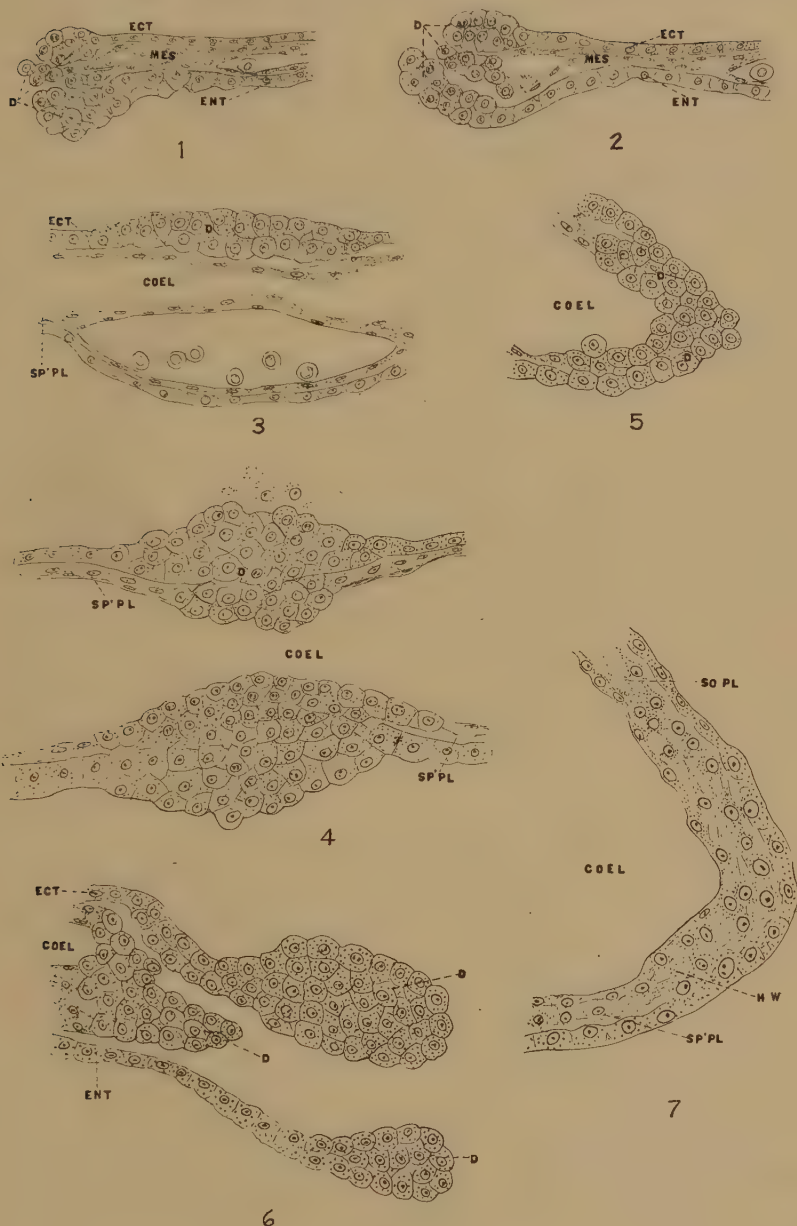


PLATE 2

EXPLANATION OF FIGURES

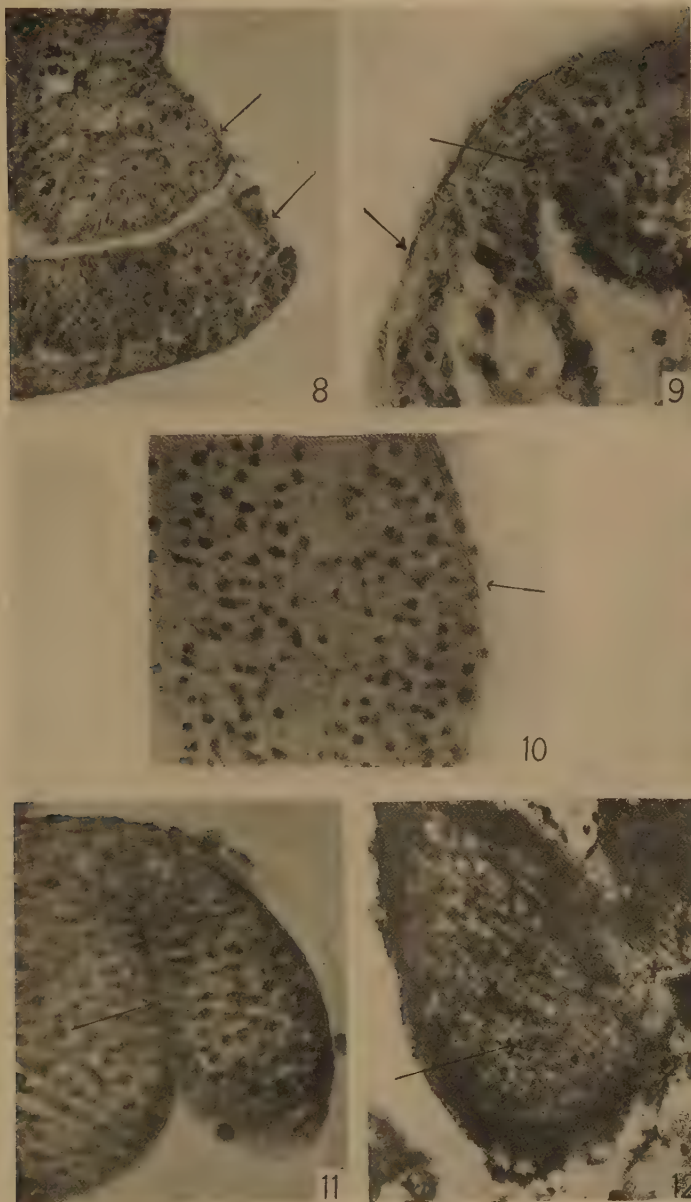
8 Photomicrograph showing transverse section of wound cutting through the splanchnopleure and somatopleure of embryo. The denuded mesenchyme has not yet been covered by the ectoderm and the coelom is not closed. Time of healing, five hours. Reduced in publication. $\times 500$.

9 Photomicrograph from experiment 54, in which healing has proceeded for twenty-four hours. Region same as figure 8. Wound is closed and covered by ectoderm, a few indifferent cells can be seen where the two mesenchymal plates join.

10 Photomicrograph of a transection of the spinal cord after twenty-four hours. The ectoderm has only partially covered the cicatrix of the cord.

11 Photomicrograph of an oblique section of a transected cord. This picture is shown to illustrate the way in which the neural canal closes for some distance beyond the wound. The cells lining the neural tube come in close contact but the line of juncture is always very distinct.

12 Photomicrograph of a cauterized wound, experiment 67, after forty-eight hours. Section is of the embryonic somatopleure and shows a large amount of dead tissue which has not as yet been eliminated.



Resumido por el autor, Ezra Allen.

Un método para la fijación de los testículos de la rata, mediante el cual se conservan los detalles citológicos así como las relaciones normales entre los túbulos y los tejidos intersticiales.

La relación normal entre el tejido intersticial y los túbulos en el testículo de la rata puede conservarse inyectando los vasos sanguíneos con el fijador "B-15" (descrito en mi trabajo acerca de los Experimentos sobre Técnica, (16)), después de expulsar la sangre con solución salina normal o con solución de Locke. Una presión de 15 a 20 mm. de mercurio es suficiente para la inyección. Para conocer la presión se une el frasco de presión con un manómetro de mercurio, comprimiendo el líquido por medio de una pera de goma, vertiendo agua gota a gota o mediante el aire comprimido. Tan pronto como los testículos se endurecen, se separan del cuerpo del animal y se colocan en el líquido fijador templado, durante treinta a sesenta minutos, al cabo de los cuales se cortan en rodajas de 2 a 4 mm. de espesor. Tanto el animal como los líquidos a inyectar deben conservarse a una temperatura de unos 38°C. durante el proceso de la inyección. La deshidratación, aclaramiento e infiltración se realizarán por cambios muy graduales de los líquidos empleados, que se verterán gota a gota. Este método fija muy bien todos los detalles citológicos, incluso los cromosomas en los estados en que tienden a aglomerarse. Otros órganos se fijan también muy bien para trabajos citológicos. Para expulsar toda la sangre de los riñones se necesitará una presión algo mayor que la indicada.

Translated by Dr. José Nonidez
Columbia University

A TECHNIQUE WHICH PRESERVES THE NORMAL CYTOLOGICAL CONDITIONS IN BOTH GERMINAL AND INTERSTITIAL TISSUE IN THE TESTIS OF THE ALBINO RAT (*MUS NORVEGICUS ALBINUS*)

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ELEVEN FIGURES

In a recent paper (Allen, '16), I described a method by which the cytological details of the germ cells in the albino rat might be demonstrated. While this method is successful for the purpose named, it does not preserve the normal relationships between the tubules and the interstitial tissue. This tissue is torn away from the tubules and distorted. These effects are shown in figures 3, 5, and 7. The normal conditions appear in figures 4, 6, and 8. Interstitial tissue in the rat testis is much less in relative quantity than in most mammals, and is so delicate that if the notoriously impermeable tunica albuginea is ruptured to admit the fixing fluid freely, the interstitial tissue is badly torn.

The purpose of this paper is to describe a method by which the normal histological and cytological relationships of the two tissues involved may be preserved. It is published with the hope that it may also be of service in suggesting a solution of similar problems in other tissues. A list of reagents and apparatus will be given and then a description of their use. An extended experience has shown that no detail may be omitted in the process without danger to the material, and for that reason the description of reagents and processes is full.

REAGENTS AND APPARATUS

Reagents

Washing fluid for removing the blood: either 0.9 per cent salt or Locke's solution.

Fixing solution:

A. Picric acid, saturated aqueous solution.....	75.0 cc.
Formal, chemically pure.....	25.0 cc.
Glacial acetic acid.....	10.0 cc.
B. Chromic acid, crystals, C.P.....	1.5 grams
Urea, crystals, C.P.....	2.0 grams

To prepare: Mix the reagents under A and warm to 38°C. in a closed vessel. Then stir in the chromic acid until completely dissolved; after which add the urea, stirring while it is being added. The resulting fluid should be transparent and rather dark brown in color. If a white precipitate forms, the difficulty is doubtless with the formalin. Ordinary commercial formalin is almost certain to produce this result. That put up by Schering never gave this trouble. The representatives of this firm are now putting out a product that seems about equal for this purpose to that formerly imported. If the solution is turbid, the fault may lie with either the formalin or the chromic acid. This latter should be as nearly equal in quality to the Kahlbaum as may be obtained. Deep red crystals have proved satisfactory.

After standing an hour or thereabouts, the fluid will turn green on account of the formation of chrome acetate, when it is not as effective for fixation as before.

Other reagents

- 5 per cent, 10 per cent, 50 per cent, and 70 per cent alcohol.
- Saturated aqueous solution of lithium carbonate.
- Anilin oil, C.P.
- Synthetic oil of wintergreen (methyl salicylate), C.P.
- 52° or 56° paraffin—the lower temperature is preferable.

The anilin oil should be nearly colorless. If doubt exists as to its purity, it should be distilled, when it will be practically colorless. A slight discoloration does not stain the tissue detrimentally.

Too great care cannot be taken to see that the chemicals are pure.

Apparatus for injection

- 1 Woulff bottle of about 500 cc. fitted with three necks and one-hole rubber corks
- 2 200-cc. bottles (preferably aspirator) for the washing and fixing fluids, fitted with perforated rubber corks.
- Either an atomizer bulb or a large supply bottle (preferably aspirator) for holding the water which will supply the pressure. If not of the aspirator type, this will need to be fitted with a siphon, and the same is true of the 200-cc. bottles.
- 1 glass U-tube.
- Rubber tubing and short pieces of glass tubing for connections.
- Clamps for rubber tubing (six usually suffice). Those of hard rubber, through which the tube passes, are most easily used. Their outline is shown in figure 1.
- One or more glass cannulae adapted to the rat's thoracic aorta, with an opening of a millimeter. Or a heart cannula, which may have an opening of 2 or 3 mm.
- 1 mercury manometer. For this a U-shaped tube may be made of small glass tubing, as shown in figure 1. The arms should be long enough to allow a movement of 20 mm. of the mercury column in each.
- A scale for reading the manometer. This is easily made from paper rules in millimeters.
- 1 thermometer.
- 1 dissecting pan.
- 1 vessel for holding warm water, through which the rubber tube carrying the injecting fluids will pass.

Apparatus for treatment of tissue after injection (Allen, 16)

- Either a mechanical agitator for agitating the fluids during dehydration, or a current of air which is passed through the fluids, thus mixing them quickly and thoroughly. This latter may be obtained from a pressure bottle (fig. 2).
- Bottle for holding the alcohols and oils. While aspirator bottles are preferable, the fluids may be siphoned through the necks of ordinary bottles.
- Two or three short tubes drawn at one end to a capillary size. These are to control the flow of alcohol and oils while they are being dropped (fig. 2).
- The rate of dropping may be controlled by a faucet, as shown in figure 2, or by the size of the capillary tube or by a plug of cotton.
- Paraffin water-bath or a carbon filament electric bulb of about 50 candle-power, which will melt the paraffin.

DETAILS OF THE METHOD

Injecting. For this purpose, the pressure-bottle apparatus rather than the syringe has been employed, since the pressure required is low, and by this method it may be measured and controlled. The Woulff bottle serves as the reservoir for compressed air. The pressure may be obtained either from a bulb, as shown in figure 1, or from a current of water flowing into the reservoir from a height sufficient to produce a pressure of 20 to 25 mm. of

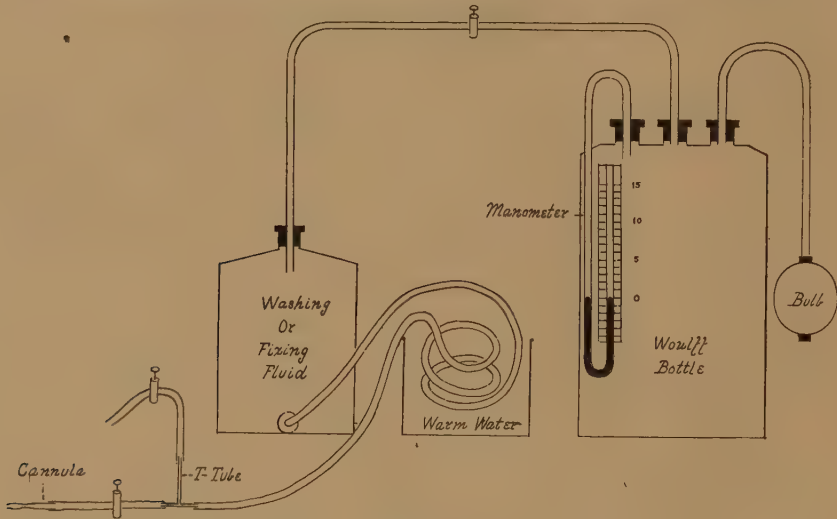


Fig. 1 Injecting apparatus. Instead of the atomizer bulb shown in this figure, pressure may be obtained from an elevated bottle of water connected by tubing to the Woulff bottle. See figure 2.

mercury against the rat's resistance. The height required is about 2 feet. This method of securing the pressure is shown in figure 2, W. B.

The injecting apparatus is shown in figure 1, which demonstrates the mode of connecting the bottles. A T-tube of glass is inserted in the delivery tube. By this means air bubbles or excess of fluid may be discharged. The rubber tube which connects the T-tube with the cannula should be as short as will permit of use. I have found that 8 inches is long enough.

Test the apparatus for freedom of flow. Avoid any air bubbles in the injecting fluids. The longer portion of the tube conveying these fluids is passed through a vessel containing warm water, so that the injecting fluid may be discharged from the cannula at about 38°C. The quantity of water should be a quart or two, and should be held at a temperature which will maintain the 38°C. point at the cannula when the injecting fluid is flowing slowly. To preserve this temperature, as well as for another reason stated below, it is desirable to have the tube between the T-tube and the cannula as short as possible.

During the process of injection the rat should be kept warm. This may be accomplished by placing the dissecting pan over a deep tray of equal size, in which hot water has been placed. Or the tray may be warmed on an electric heater.

When all is in readiness, the rat is lightly anesthetized by either chloroform or ether. It is important that the heart be beating or that it has just stopped beating, as in many cases a brief delay after the heart stops seems to interfere with the free passage of the washing fluid at the low pressure employed. I have injected through the thoracic aorta rather than the heart. To expose this vessel, an incision is made from the penis to the anterior ribs, severing the ribs a little to the left of the sternum. A second incision is made at right angles to the first, just posterior to the ribs, and the diaphragm cut on the left side so that the thorax on that side may be thrown open by bending the ribs back and breaking them. After removing the slight amount of fat about the artery, the cannula is inserted, care being taken that a slight flow of the washing fluid is maintained in order to prevent air bubbles. As soon as the cannula is secured in place, I increase the pressure gradually but quickly until the vena cava is well filled at a part just anterior to the liver. The vena cava is then cut at this point and the pressure turned on full—20 to 25 mm., counting the sum total of the movement in both arms. As washing proceeds, I watch the intestines and liver. Usually by the time the former are cleared of blood and the latter is beginning to pale, the testes have been thoroughly washed out. It is well, however, as a final test, to examine the testes by pulling

them into the body cavity (if not already retracted), and noting whether they are white. During the injection of both the washing and fixing fluids it seems advantageous to let the testes lie within the scrotum.

When washing is seen to be complete, the tube is changed from the washing bottle to that containing the fixing solution. The excess of washing fluid is washed out of the discharge tube through the arm of the T-tube designed for that purpose, as previously noted, without removing the cannula from the artery. At the same time any air bubbles which may have entered with the fixative may be passed out by the same channel. During this changing, the clamp between the T-tube and the cannula has been closed. As soon as this clamp is removed the fixative will begin to flow toward the animal. This fluid will mix in the short tube between the T-tube and the cannula, but since its capacity is so small the fixing fluid at full strength almost immediately replaces the washing fluid, a very important consideration if good fixation is to be secured.

If the rat is large, the fixative should flow until about 100 cc. has been used. A less quantity will be sufficient for smaller rats. The picric acid quickly changes the color of the feet and the intestines, so that the progress of the fixative is easily observed. It is well to let the flow continue until the testes feel quite hard, which will usually be their condition after about 75 or 100 cubic mm. of fixing fluid has passed through. As soon as hard, the testes are removed from the body without cutting the tunica albuginea.

HARDENING AND DEHYDRATION

The subsequent steps are as important as the process of injection. Injection has been employed to secure quick and thorough distribution of the fixative throughout the organ without rupturing the tunic. The next problem is to maintain the fixation through the subsequent changes of fluids. The connective tissue holding the tubules together is so delicate that it is very easily torn by sudden changes. By proceeding from one fluid to another very gradually, this disaster is avoided. One method is described below. Others perhaps simpler may be found later.

To complete the hardening of the testes, they are placed immediately upon removal from the body in fresh fixative at 38°C., where they remain at that temperature for thirty minutes, after which they are cut into pieces of about 4 mm. thick or less by the blade of a safety razor, and the process of dehydration begun.

This is carried on by the drop method. That is, the fluid to be added is transferred drop by drop to the fluid in which the tissue lies. By keeping the mixture thus produced in constant motion,

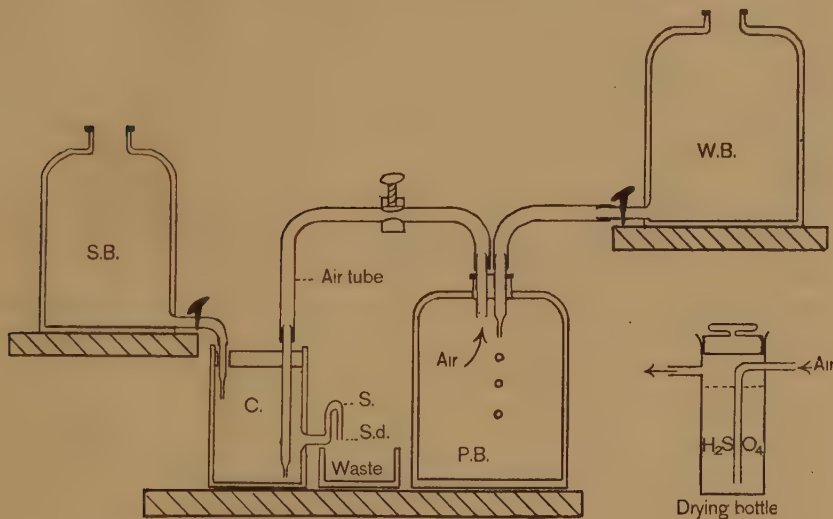


Fig. 2 Dehydrating apparatus. P.B., pressure bottle; S., siphon; S.d., siphon discharge; S.B., supply bottle for alcohol or oil; W.B., water bottle; C., container for tissue. See Allen ('16).

these drops are rapidly and intimately mixed without exposing the tissue to a sudden difference of density or flow of fluids. The agitation is so gentle that the tissues need not be moved by the currents. (Allen, '16.)

Dehydration is accomplished by the following steps: from the fixing solution the tissue may be transferred directly to 5 per cent alcohol without injury. It may remain in this fluid for 45 minutes, after which the dropping of the 10 per cent alcohol into the 5 per cent is started. To this 10 per cent alcohol a small

quantity (say, 1 per cent) of a saturated solution of lithium carbonate has been added. This salt hastens the removal of the picric acid. After the mixture has reached a strength of 10 per cent alcohol, it may remain for one or two hours, agitation being maintained, and a few drops of lithium carbonate solution may be added from time to time by a pipette; or the mixture of 10 per cent alcohol and lithium carbonate be dropped in as previously, but very slowly—one drop in ten seconds. At the expiration of the two hours, change the tissue into fresh 10 per cent alcohol and begin dropping in 50 per cent alcohol and about 1 per cent lithium carbonate solution, about one drop per second, until enough of the mixture is added to bring the fluid up to 30 per cent alcohol, and in this strength the tissue should remain for about an hour.

Determination of the strength of alcohol in which the tissue will lie after such a dropping process may be made by estimating the quantity of the new fluid, to be added to that of the lower percentage, which will be required for the purpose, and this quantity placed in the supply bottle.

From the 30 per cent alcohol, the tissue is to pass to a mixture of equal parts of 50 per cent alcohol and anilin oil. By experiment it has been found that this is as high a concentration of anilin as will mix well with 30 per cent alcohol. This mixture should be added in very small drops and at the rate of not more than one in five seconds. If a precipitate appears after the flow has continued for a few minutes, it is a sign that the fixing fluid has not been entirely removed from the tissue, which must then be returned to the 30 per cent alcohol and lithium carbonate for a longer period.

When the new mixture reaches the stage of equal parts of anilin and 50 per cent alcohol the next fluid should be started, which is equal parts of anilin and 70 per cent alcohol, dropped as slowly as before. When the new mixture reaches the stages of this last fluid, the tissue is changed to fresh 70 per cent and anilin, and pure anilin is started dropping at the rate of about one drop in ten seconds. If the drop is reduced to the minimum in size, the rate may be one in five seconds. The anilin is so much heavier than

alcohol that the exchange must take place very slowly. It may well require twelve to fourteen hours, so that this part of the process may take place during the night. Dehydration will be complete when the tissue is clear like amber and has been passed through one change of pure anilin.

It should be stated that at the time of changing from one fluid to another higher in alcohol or anilin content, the tissue should be placed in fresh fluid of the strength to which it has arrived, as stated definitely in the step from 5 per cent alcohol to 10 per cent, and again in the step to anilin.

CLEARING AND INFILTRATING WITH PARAFFIN

The clearing oil should be added by the same method as that used for the alcohol and anilin. It is important to remove all of the anilin from the tissue in order that the paraffin may infiltrate thoroughly. For clearing, I have found the synthetic oil of wintergreen more satisfactory for the rat testis than any other clearing agent, although the cedar-wood oil is also very excellent.

The mixing of paraffin with any clearing oil is difficult if the necessary shrinkage of the specimen incident thereto is to be gradual. For this reason the paraffin must be added slowly. I have found that carefully graduated strengths of a mixture of the oil and paraffin give very satisfactory results. The steps employed have been eight, beginning with 10 per cent melted paraffin in wintergreen oil, then 20 per cent, 30 per cent, 40 per cent, 50 per cent, 60 per cent, 80 per cent, 90 per cent, and finally pure paraffin, keeping the tissue warmed to the necessary temperature during these changes. It is better to err on the side of too gradual than too sudden a progress. Not less than two hours should be consumed in this process. Four or five changes of paraffin, requiring two or three hours, are then necessary to remove all traces of the oil.

Up to the present time I have used no method of agitating the oil during the addition of the paraffin, and consequently have not attempted to employ a dropping mechanism in the paraffin oven. The oil of wintergreen is so much heavier than melted paraffin that it does not mix readily without agitation. A dropping and

agitating mechanical device might be devised for working at a temperature required for melted paraffin.

IMBEDDING AND SECTIONING

The remaining steps of imbedding and sectioning are carried on as usual. The sections should be spread to the limit by heating them to a degree just below the melting-point. Overhead electrical heat is preferable to the flame for this purpose. It is easily employed by using a rather high-power carbon filament bulb (50 candle-power) in a reflector. If the slides are supported on glass rods or other small framework rather than laid directly upon the table, the paraffin spreads more slowly and there is very little danger of overheating the material. A temperature control is readily supplied by an extra slide upon which some of the waste paraffin sections are floated. When spreading is thus complete, the water should be drained off and the sections oriented if necessary. While drying, the slides should be kept as warm as possible without melting, in order to prevent the slight shrinkage of tissue which occurs if the slides dry cold. Both the spreading and drying may be carried in paraffin ovens properly regulated in temperature for each procedure.

RESULTS

The figures show that by the method described the delicate interstitial tissue is retained in its normal position attached to the limiting membranes of the tubules: that the cytological details of the cells in both tubules and interstitial tissue are also retained.

In normal rats no extravasation has been observed, nor stretching of the vascular walls. In some rats in poor condition extravasation has occurred.

If the washing fluid is run for a longer time than necessary to wash out the testes, each of the internal organs in the abdominal cavity will be freed from blood and may be used for cytological purposes. The kidneys may require a little higher pressure. The method preserves the wall of the alimentary canal very well, preserving with extreme delicacy the central portion of the villi.

With this tissue it is well to remove a portion of the intestine while the low pressure is still on and inject the lumen with the fixing fluid by means of a pipette, and then drop the piece into the fixing fluid and treat as described for the testis. The division figures in the mucosa cells are thus very well differentiated.

LITERATURE CITED

- ALLEN, E. 1916 Studies on cell division in the albino rat. II. Experiments on technique, with description of a method for demonstrating the cytological details of dividing cells in brain and testis. *Anat. Rec.*, vol. 10.

PLATE 1

EXPLANATION OF FIGURES

The figures are photomicrographs made with a Zeiss projection ocular, a Watson condenser, and objectives chosen according to the magnification desired. They were all reduced one-third in reproduction. The sections were cut at 7μ .

3 and 5 Sections of testis fixed by cutting the organ into small pieces with scissors. Stained with iron haematoxylin. $\times 47$.

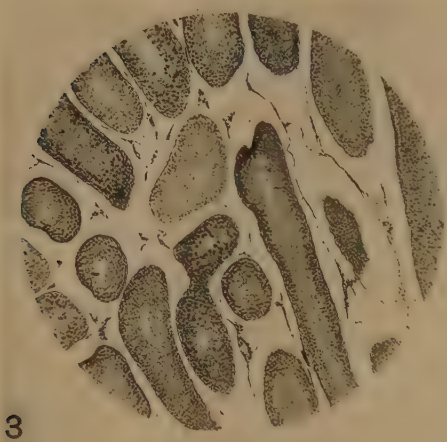
4 and 6 Sections of testis prepared by the method here described. Stained with iron haematoxylin and acid fuchsin. $\times 47$.

7 Portion of interstitial tissue from the same section as figure 3. Stained with iron haematoxylin. $\times 667$.

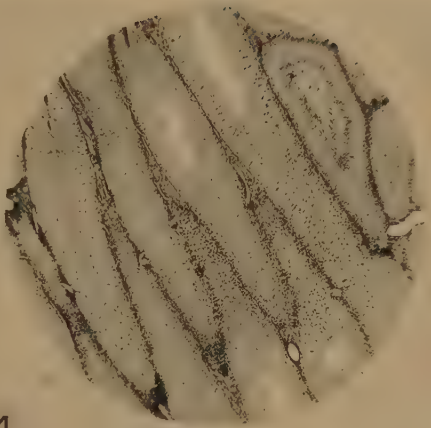
8 Interstitial tissue from same preparation as shown in figures 4 and 6. The glandular and endothelial nuclei are prominent. The dark line running up and down in the figure is the membrana limitans out of focus. The interstitial takes the stain much more deeply than the germinal tissue. Stained with iron haematoxylin and acid fuchsin. $\times 667$.

9 Germinal tissue, showing chiefly first spermatocytes in late prophase and metaphase. From same section as figures 4 and 6. $\times 333$.

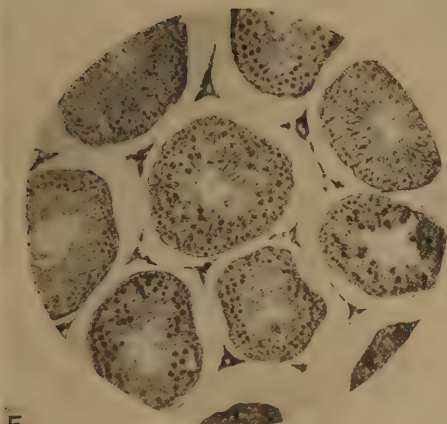
10 and 11 Two cells from same portion of tissue shown in figure 9 photographed under the oil-immersion lens to show fixation of the chromosomes in metaphase. $\times 667$.



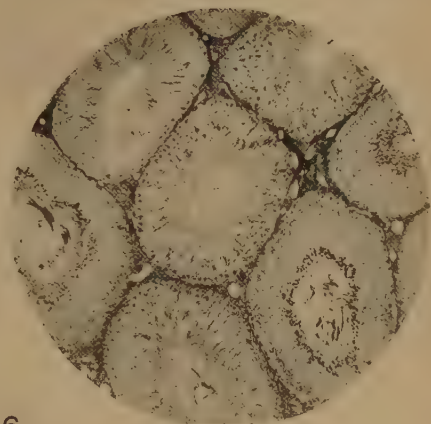
3



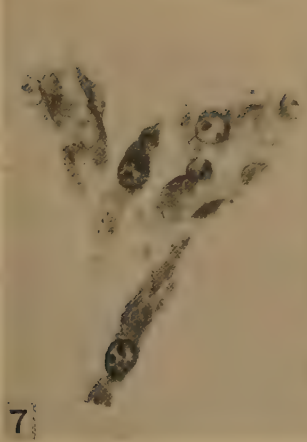
4



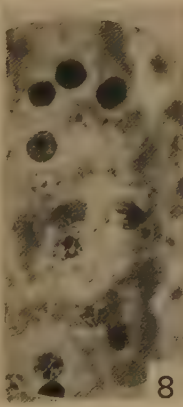
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11

Resumido por el autor, Harrison H. Hunt.

Las variaciones de la vena tiroidea inferior del gato doméstico.

La vena tiroidea inferior vierte la sangre que conduce en cualquiera de las siguientes venas: innominada izquierda, yugular interna izquierda, innominada derecha, yugular externa derecha, yugular interna del mismo lado y precava. En uno de los casos la vena tiroidea inferior se dividía en su extremo posterior en dos ramas una de las cuales entraba en la vena innominada derecha, la otra en la izquierda. Próximamente en la mitad de los gatos estudiados la tiroidea inferior se vertía en la innominada izquierda. En su extremo anterior la vena en cuestión, generalmente, aunque no siempre, se divide dicotómicamente. Generalmente esta ramificación está situada entre los lóbulos de la glándula tiroides, aunque en algunas ocasiones, se presenta en el mismo nivel que el extremo anterior de la glándula tiroides o posteriormente a dicha glándula.

Translated by Dr. José Nonidez
Columbia University

THE VARIATIONS OF THE INFERIOR THYROID VEIN OF THE DOMESTIC CAT

HARRISON R. HUNT

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SEVEN FIGURES

The inferior thyroid veins of man vary considerably. The following observations, made on thirty-three domestic cats selected at random, show that the same is true of this vein in the cat. Each of the accompanying figures represents, somewhat diagrammatically, the conditions in a single animal. These seven animals suffice to give a fairly complete idea of the variations in the remaining twenty-six.

Figure 1 shows the inferior thyroid vein (1) communicating anteriorly with the left internal jugular, receiving branches from only the left lobe of the thyroid gland (14), then passing obliquely backward across the trachea (13) to join the right innominate vein (10). The veins labeled 2 and 3 in this and the following figures were not homologized with certainty with the human superior and middle thyroid veins. Two other dissections resembled figure 1 very closely, except that in one of them the inferior thyroid vein joined the external jugular vein at *a*.

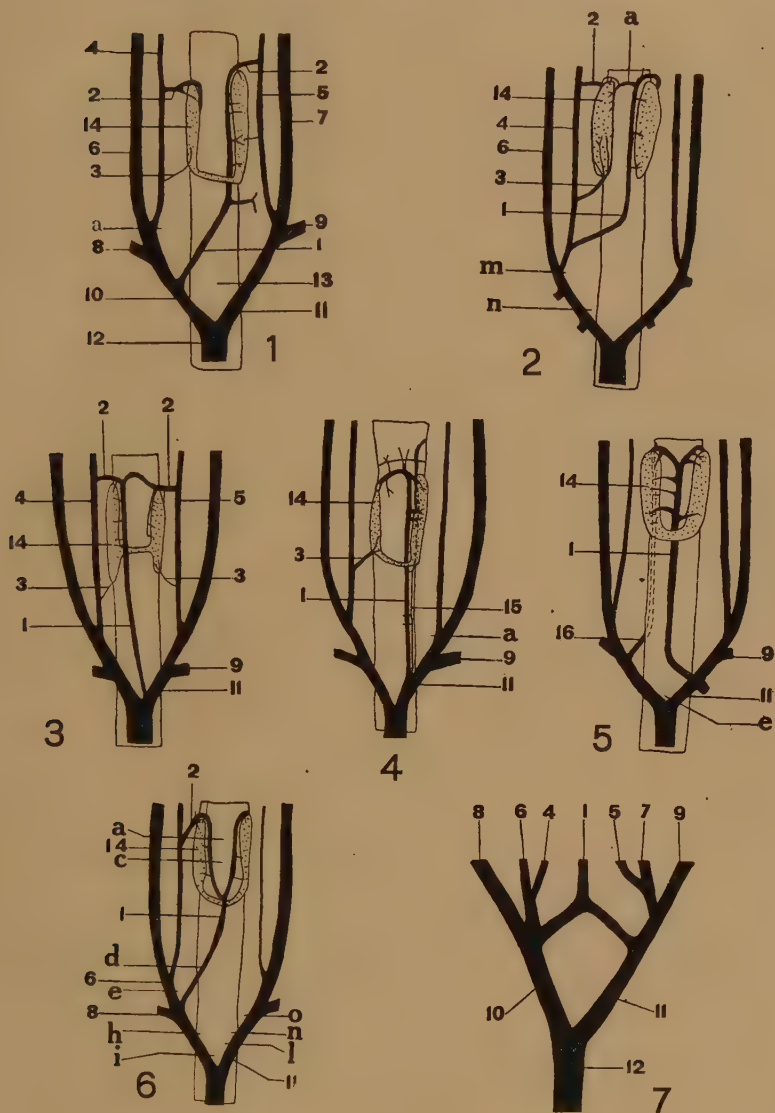
Figure 2 resembles figure 1 in some respects. However, the inferior thyroid vein (1) in figure 2 receives a branch (*a*) from the right lobe of the thyroid gland (14), empties into the right internal jugular vein (4), but in the dissection did not appear to communicate directly with the left internal jugular. The inferior thyroid vein in a second cat joined the jugulars at *m*

(fig. 2), and its transverse branch (fig. 2, *a*) could not be traced as far as the right lobe of the thyroid gland. In a third individual, vessels *a* and 3 (fig. 2) were connected by a conspicuous vessel running along the dorsal surface of the right lobe of the thyroid gland, and the inferior thyroid vein entered the right innominate at *n* (fig. 2). In all other respects the inferior thyroid veins in these three cats were very similar.

In one case (fig. 3) the inferior thyroid vein (1) was formed by the union of a branch (2) from each of the internal jugular veins (4 and 5). The inferior thyroid, after receiving branches from the right lobe of the thyroid gland (14), passed obliquely backward, joining the left innominate vein (11) near its union with the precava.

The resemblance between the inferior thyroid veins (1) in figures 3 and 4 is evident. However, in figure 4 the inferior thyroid apparently was unconnected, at its anterior end, with the internal jugular veins; it lay close to the left lobe of the thyroid gland; and its junction with the left innominate vein was considerably anterior to the junction shown in figure 3. A small vein (fig. 4, 15) ran along the dorsal side of the left lobe of the thyroid gland parallel to the inferior thyroid vein (1), connecting anteriorly and posteriorly with the latter vessel. One other cat showed practically the same conditions as those represented in figure 4, though the vein 15 (fig. 4) was not found, and the inferior thyroid vein emptied into the innominate at *a* (fig. 4).

In figure 5 the two vessels uniting to form the inferior thyroid vein (1) passed backward a short distance before coming together. The inferior thyroid vein was median in position, receiving small side branches from both lobes of the thyroid gland (14). Except for the fact that 16 and 15 (fig. 4) lay on opposite sides of the trachea, their locations and courses were quite similar. In four other individuals the inferior thyroid vein strongly resembled the same vein in figure 5, though in one case it joined the precava at *e* (fig. 5).



EXPLANATION OF FIGURE NUMERALS

1, inferior thyroid vein; 2 and 3, branches of the internal jugular veins; 4 right internal jugular vein; 5, left internal jugular vein; 6, right external jugular vein; 7, left external jugular vein; 8, right subclavian vein; 9, left subclavian vein; 10, right innominate vein; 11, left innominate vein; 12, precava; 13, trachea; 14, thyroid gland. (For the significance of 15, 16, and the letters, see text.)

In the animal from which figure 6 was drawn a vein ran backward near the medial margin of each lobe of the thyroid gland (14), the two veins uniting at the level of the isthmus of the gland. One of these vessels (2) branched off from the right internal jugular vein. The inferior thyroid vein (1) emptied into the innominate where the external jugular and subclavian veins joined (8). The variations of the inferior thyroid vein in thirteen more cats can best be described by reference to the lettering in figure 6. In twelve of these animals the vein branched at approximately the following points: in one case at *a* (the branching thus closely resembling the branching of the inferior thyroid vein in fig. 5), in four cases at *c*, in six animals at the place where the inferior thyroid vein branches in figure 6, and in one animal as far back as *d*. The approximate points at which the inferior thyroid vein in these thirteen cats emptied into the innominate and external jugular veins varied greatly (fig. 6). In four cases this union was at *o*, in four cases at *n*, in two at *l*, and in three cases at the points *e*, *h*, and *i*, respectively. In two of these individuals the left anterior branch could be traced to the left internal jugular vein; in two cases, including the animal shown in figure 6, the right anterior branch came from the right internal jugular vein.

Incomplete records of the inferior thyroid veins in four animals show that the vein emptied on the right side in three of them, and into the left internal jugular in the fourth animal.

Figure 7 represents the posterior end of the inferior thyroid vein (1) in one animal. The anterior portions of the vein were not drawn. The vessel divided into two branches which joined the innominate veins (10 and 11) at the places shown in the figure.

Possibly some very small veins emptying into the inferior thyroid vein were not well injected in all the cats examined, thus escaping observation. This might explain the failure in many cases to find connections between the inferior thyroid and internal jugular veins near the anterior end of the thyroid gland.

TABLE 1

	NUMBER OF CATS
Inferior thyroid vein unbranched at its anterior end.....	3
Inferior thyroid vein divided into two branches at the anterior end of the thyroid gland.....	6
Inferior thyroid vein divided into two branches at varying positions between the lobes of the thyroid gland.....	17
Inferior thyroid vein dividing into two branches posterior to the thyroid gland.....	1

TABLE 2

	NUMBER OF CATS
Inferior thyroid vein emptying into left innominate.....	17
Inferior thyroid vein emptying into the left internal jugular.....	1
Number of cases in which the vein emptied on left side.....	18
Inferior thyroid vein emptying into right innominate.....	6
Inferior thyroid vein emptying into right external jugular.....	3
Inferior thyroid vein emptying into right internal jugular.....	1
Inferior thyroid vein emptying on the right side (exact position not noted)	3
Number of cases in which the vein emptied on the right side.....	13
Inferior thyroid vein emptying into precava.....	1
Inferior thyroid vein emptying into both innominate veins.....	1

SUMMARY

The results of this investigation may be summarized best in tabular form. Table 1 shows the variation in the dichotomous branching of the inferior thyroid vein at its anterior end. Table 2 summarizes the variations of the point at which the vein emptied posteriorly into the larger venous trunks.

Thus the inferior thyroid vein in the majority of cases branches between the lobes of the thyroid gland and enters the larger veins on the left side.

THE ANATOMICAL RECORD, VOL. 16, NO. 2
APRIL, 1919

Resumido por el autor, Eben James Carey.

Estudios teratológicos.

A. Sobre un phocomelus, con especial mención de las extremidades. El carácter principal de esta clase de monstruos es un acortamiento anormal y cesación del desarrollo de algunos o todos los huesos largos de las extremidades. El presente estudio revela el hecho de que en ausencia completa o desarrollo rudimentario de una parte del esqueleto, se encuentra también una falta completa o parcial de los músculos relacionados con él. Lo inverso es también cierto, pues un excesivo desarrollo de las partes esqueléticas está acompañado por un grado mayor de desarrollo en los músculos relacionados con ellas. B. La forma externa de un embrión humano anormal de veintitrés días. El autor da una detallada descripción de la forma externa de este embrión y una descripción de la interesante malformación de la región cervical. El estado de desarrollo de la forma exterior coloca a este embrión entre el descrito por Bremer, de 4 mm. de longitud y 21 días de edad, y el descrito por Mall, de 7 mm. y 26 días, de modo que la edad probable del que se describe es 23 días. C. y D. Las anomalías de los monstruos anencefálicos: cranioraquisquis completa. El hecho más interesante con relación a los monstruos anencefálicos, ya notado por varios observadores, es que generalmente pertenecen al sexo femenino. Las anomalías bien marcadas, que se describen en los presentes estudios, son: la falta de cerebro, generalmente también de médula espinal y la falta de desarrollo de los huesos que integran la bóveda craneal y la lámina de la columna vertebral. La boca típicamente abierta de los monstruos anencefálicos está relacionada con la falta de la bóveda craneal y la pérdida correspondiente de la porción anterior del músculo temporal. Las disecciones revelan también la existencia de una interrelación definida entre el desarrollo de los tejidos óseo y muscular.

TERATOLOGICAL STUDIES

A. ON A PHOCOMELUS, WITH ESPECIAL REFERENCE TO THE
EXTREMITIES

B. THE EXTERNAL FORM OF AN ABNORMAL HUMAN EMBRYO OF
TWENTY-THREE DAYS

C. THE ANOMALIES OF AN ANENCEPHALIC MONSTER.
COMPLETE CRANIORRHACHISCHISIS

D. A SECOND ANENCEPHALIC MONSTER. COMPLETE
CRANIORRHACHISCHISIS

EBEN J. CAREY

Department of Anatomy, Creighton Medical College, Omaha, Nebraska

SEVENTEEN FIGURES

ACKNOWLEDGMENTS

I wish to express my sincere thanks to Drs. Alonzo Mack, J. S. Foote, T. J. Dwyer, and C. J. Nemec, for the specimens herein studied, and to Dr. A. F. Tyler, for the skiagraphs of the skeletons, and would also acknowledge the helpful interest and suggestions of Prof. H. von W. Schulte, Director of the Department of Anatomy in this school.

A. ON A PHOCOMELUS WITH ESPECIAL REFERENCE TO THE
EXTREMITIES

The term phocomelus is derived from the Greek $\phi \acute{o} \chi \eta$, seal, and $\mu \acute{\epsilon} \lambda \omicron \varsigma$, limb. The chief characteristic of monsters belonging to this class is an abnormal shortening and arrest of development of some or all of the long bones of the extremities. The feet and hands are usually composed of the normal number of skeletal elements, but generally appear to arise directly from the

pelvic and shoulder-girdles, respectively, which lends them the fantastic appearance of a seal's flippers.

The specimen described was obtained by Doctor Mack, Professor of Obstetrics, Creighton Medical College, February, 1917. It was a full-term, still-born fetus, and parturition was marked by excessive dystocia. The weight was 2500 grams and the crown-rump measurement 25 cm. Through the shoulder and pelvic regions it measured 12 and 10 cm., respectively.

External form

The marked umbilical hernia, in which the coils of the intestines show through the attenuated walls at the base of the umbilical cord, is readily apparent in figure 1. This is due to arrested development. Normally, five or six primitive intestinal loops, by rapid elongation in embryos between 17 and 20 mm. in length, push their way into the umbilical coelom, producing the normal hernia funiculi umbilicalis physiologica, where they remain until the embryo reaches a length of between 35 and 45 mm. Soon after the latter period the intestinal loops return to the abdominal cavity proper.

The lateral contour of the abdominal and thoracic regions is exceedingly convex, depending upon the excessive size of the liver. Normally, the liver at term is one-twentieth of the total body weight in the still-born, Jackson ('09). The liver in this case, however, weighs 490 grams, or about one-fifth of the total body weight. The excessive size of the liver is due to two factors: first the umbilical hernia; second, the cleft sternum, both operating to release the liver from the confinement which is normally present. The liver in coelossomic monsters, like the brain in cases of hydrocephalus, shows a tendency to assume an unnatural bulk when relieved from the normal pressure of relational structures.

In the embryo the ventral wall of the trunk is at first very thin, and the heart with its various parts as well as the liver and other viscera may be seen through it. From the sides the anlagen of the skeleton and musculature grow into the walls: arrest of this growth may occur and produce ectopia cordis and fissura sterni,



Fig. 1 Phocomelus, ventral view.
 Fig. 2 Phocomelus, right lateral view.
 Fig. 3 Phocomelus, left lateral view.
 Fig. 4 Phocomelus, dorsal view.

which depend upon the disturbance in the thoracic region. A portion of the intestine, as noted above, normally projects into the umbilical cord at the time that the outward growth of the abdominal walls occurs. Normally, the intestinal hernia recedes into the abdominal cavity with the further development of the abdominal walls, but if the latter are arrested in their development, the hernia persists, as is seen in this specimen.

The upper extremities are much distorted and resemble in no way, except in the contour of the shoulder and in the digits, the extremities of a normal full-term fetus. The palmar surfaces of the hands are turned mesiad, a retention of the position which is normal in embryos of 18 to 25 mm. in length.

The median raphe extending from the anus to the scrotum is well marked. The scrotum is composed of two fat-filled pouches which do not enclose the testicles. These organs are found in the inguinal canal about ready to emerge at the external abdominal ring.

The lower extremities also show the degree of rotation characteristic of a normal embryo 18 to 25 mm. in length. The knees are directed ventrolateral, while the plantar surfaces of the feet are turned mesial and consequently are opposed to each other. The dorsum of each foot is unusually high. The abducted position of the great toe and the metatarsal pads are especially clearly seen in the right foot. This is characteristic of embryos of about 25 mm. in length. The metatarsal pads normally undergo a gradual retrogression and their outlines become indistinct during the fourth and fifth months. The phalangeal pads of the great toe, and in a less degree the second and third toes, of the right foot are clearly shown.

The right upper extremity is best seen in figure 2. The shoulder and wrist are recognizable and a slight groove dorsally in the region of the axilla marks the position of the elbow. The limb is so placed that the extensor surface is directed laterad. The five digits are distinct; the thumb is certainly rudimentary. The hand is unusually broad toward the base of the fingers; this is another early fetal characteristic which has been retained.

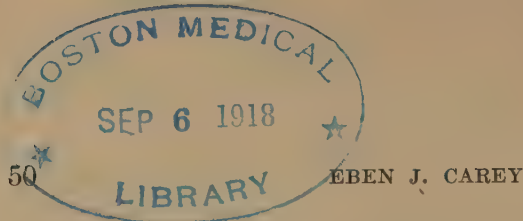
In the lower extremity the heels are well marked and the foot is extended as a result of the contracture of the gastrocnemius muscle; this occasions the talipes equinus variety of club-foot. In addition, the foot is inverted so that the soles are opposed to each other by the contracture of the tibialis anticus muscle, resulting in the talipes varus variety of the club-foot. This is merely arrested development, however, for the position described above is a normal phase of limb rotation and is regularly present in embryos of 35 mm.

The head is abnormally large; the forehead is especially protuberant. This is partly due to arrested development and partly to its hydrocephalic condition. The anterior and posterior fontanelles and sagittal suture gape in an abnormal manner because of the distended cerebral hemispheres. The protuberant forehead resembles the condition found in prosencephalic monsters. In addition to hydrocephalus, there is an extensive meningeal hemorrhage incident to labor.

The root of the nose is deeply depressed, and the nose as a whole is very low and broad. The upper lip projects, whereas the lower one recedes. The depression between the root of the nose and forehead is normal in embryos between 18 and 42 mm. in length, but later this character is effaced.

The shoulders are distinctly marked, as seen in figure 3. The protuberance due to the acromial process of the scapula is especially distinct on the left side. The disproportion between the segments of the limbs is striking. In each lower limb a dorsal groove is seen which corresponds to the popliteal space. It is at once evident (fig. 4) that the region of the thighs is greatly shortened, and a corresponding shortening of the proximal segment of the upper limb is also apparent.

The left lateral aspect is reproduced to show the symmetry of the surface abnormalities (compare figs. 2 and 4). Externally a tendency is detectable to subdivision of the upper extremity into arm, forearm, and hands by grooves which limit these regions. Rudiments of the finger nails may be detected, but these structures have not broken through the overlying epidermis.



In the lower extremity the regional outlines are not as distinct as in the upper. The area corresponding to the knee is directed cephalolateral, but there is no definite demarcation between the dorsum of the foot and the leg. The toe nails and toes are not as well developed as the finger nails and the fingers.

The rotundity of the cheeks is quite marked (fig. 4), and well-developed sucking pads were found on dissection.

The symmetrical arrest of development of the lower extremities is clearly seen in figure 5.

Internal anatomy

The section of the alimentary canal, contained in the umbilical hernia, consists of the cecum, appendix, 2 cm. of the ascending colon, and 10 cm. of the ileum. The latter possesses a marked Meckel's diverticulum, from the apex of which a fibrous strand extends into the cord for some distance, eventually to be lost in its connective tissue. The remaining coils of the small intestine are arranged in the normal manner. Coil no. 1 forms the duodenum; the secondary derivatives of coils nos. 2 and 3 are found in the left hypochondriac region, those of coil no. 4 are found in the right hypochondriac region, while those from coil no. 5 are located in the left iliac fossa. So far the arrangement of the small intestine is normal; however, the derivatives of coil no. 6, instead of occupying the hypogastric region, extend directly to the base of the umbilical cord and enter into the umbilical hernia as noted above.

The liver is nearly three times the size of the organ normally found in still-born infants by Jackson ('09), and the spleen is nearly six times its normal weight. The kidneys and spleen are also found to be overweight. The pancreas, bladder, prostate, and testicles are about the normal size.

The heart is hypertrophied; it is about double the average size. The two lungs are much compressed, being only of about one-half the normal size. The thymus is double the average weight, whereas the thyroid is about normal. To facilitate comparison with conditions in still-born infants of the tenth month, the



Fig. 5 Phocomelus, caudal view.

Fig. 6 Phocomelus, skiagraph from in front. Natural position of limbs.

Fig. 7 Phocomelus, skiagraph. The arms abducted to show curvature of the radii.

Fig. 8 Phocomelus. Skeleton of the upper extremity.

TABLE 1

Relative sizes of the fetal organs of the still-born phocomelus compared to those in still-born infants by Jackson

	MALE STILL-BORN TENTH MONTH (JACKSON)		MALE STILL-BORN PHOCOMELUS TENTH MONTH. TOTAL WEIGHT 2500 GRAMS	
	Cat. number	Per cent	Weight.	Per cent
			<i>grams</i>	
Brain.....	71	12.91	450.00	18.00
Thymus.....	65	0.296	12.5	0.5
Heart.....	80	0.69	30.0	1.2
Right lung.....	69	0.98	10.0	0.4
Left lung.....	69	0.79	10.0	0.4
Liver.....	71	4.81	300.0	12.2
Spleen.....	70	0.27	40.0	1.6
Right kidney.....	9	0.367	25.0	1.0
Left kidney.....	9	0.341	25.0	1.0
Right suprarenal.....	2	0.101	3.0	0.125
Left suprarenal.....	2	0.111	3.0	0.125
Thyroid.....	26	0.111	4.0	0.16

weights of the several organs and their percentage of the total body weight are given in table 1, to which are added the findings of Jackson in his series of still-born infants.

Skeletal and muscular systems

The scapula is peculiar in that it possesses no glenoid articular cavity; in its place there is a large rounded protuberance which fits into a corresponding cartilaginous depression of the humerus. The body of the scapula is ossified, but the vertebral border, inferior angle, coracoid process, and acromion are cartilaginous.

A supraspinous muscle arises from the corresponding fossa, but no omohyoid nor levator anguli scapulae muscles are present. The trapezius and rhomboid muscles are normally located. From the infraspinous fossa arises an infrapinatus and from the vertebral border the teres major and minor take origin. The fibers of the deltoid and trapezius muscles are, for the most part, directly continuous over the spine of the scapula, but there

is a deep fibrous inscription which unites this complex muscle mass to the spine. There is a well-marked cartilaginous supraglenoid tubercle for the attachment of the long head of the biceps; the short head of this muscle arises in common with the coracobrachialis muscle from the coracoid process. Both heads of the biceps, the coracobrachialis and the deltoid muscles, fuse to form one large complex which is inserted into the radius, no fibers whatever finding attachment on the humerus. From the infraglenoid tubercle, which is cartilaginous, arises the middle or long head of the triceps; this is the only representative of the normal triceps muscle, the other two heads being absent.

The humerus is represented by a small, all but shapeless mass of soft cartilage. On its superior aspect it presents a deep articular cavity into which fits the head on the scapula described above. This head is firm and calcified, but no secondary ossification center is present; absolutely no calcification is found in the humeral mass. No doubt this difference in density is the immediate cause for the reversal of curvature at the shoulder-joint, determining the presence of a scapular head and a humeral articular cavity. Caudal to this cavity the humerus is produced into a rounded process of cartilage surrounded by a dense mass of fibrous tissue, upon which is inserted the teres major and minor, supraspinatus and pectoralis minor muscles. No brachialis anticus muscle is present. A few fibrous strands extend from the pectoralis major and latissimus dorsi, to the dense perichondrium of the humeral mass, but the major part of the insertions of these two muscles are by tendon into the proximal end of the radius.

The common origin of the flexor group of muscles of the forearm, for the most part, is from the proximal end of the radius. There is a small direct continuity, however, on the part of the flexor muscles with the biceps by means of muscular slips. Similarly, the extensor group, arising in the main from the proximal end of the radius, has direct muscular continuity with the triceps.

The radius is the largest and longest bone of the upper extremity. It is well ossified and bowed in adaptation to the abnormal stresses and strains to which it is subjected. At its upper end it presents a concavoconvex articular surface which artic-

ulates with the lower convex surface of the humerus. The ulna does not enter into the formation of the elbow-joint and is merely a cartilaginous bar extending from the upper extremity of the radius to the carpus.

The carpal elements, navicular, lunate, triquetral, pisiform, greater multangular, lesser multangular, capitate and hamate, are each definable. They are, however, nothing but cartilaginous nodules presenting but a very faint resemblance to the normal components of the carpus.

In the metacarpus and phalanges the normal number of elements are present, but they are abnormally shortened, especially the metacarpals. All are in a cartilaginous state except the terminal phalanges in which ossification is beginning at the distal extremities. The proximal articular ends of these phalanges are cartilaginous.

The wrist is extended and the hand is adducted towards the ulnar side. It is interesting to note at this point that the flexor carpi ulnaris and the extensor carpi ulnaris are practically one muscle. At their origins these muscles are inseparable. The former is inserted partly into the pisiform and partly into the ulnar side of the base of the fifth metacarpal element. The latter muscle is inserted also, on the ulnar side of the base of the fifth metacarpal element. The greater muscular mass of the extensor group combined with the physiological unity of the flexor and extensor carpi ulnaris muscles explains the extended position of the wrist and the inclination of the hand to the ulnar side.

The sternum is widely cleft, indeed, union is present only at its cephalic extremity. This point evidently represents the persistent episternal band which has become chondrified. Through this band the clavicles are in direct continuity across the ventral median line. The extent of development of the thoracic walls is comparable to that of an embryo of about 17 mm. in length (Muller, '06). The sternal ends of the lower eight cartilaginous ribs do not extend medialward beyond the midaxillary line, and in this connection it is interesting to note that both the recto-abdominales are absent. It is highly probable that their development was initiated, but that subsequently they degenerated, for

a rectus sheath was found on dissection containing a mass of adipose tissue. The oblique and transversalis are imperfect especially towards the thorax. The nerves of the region appear normal and have the usual course and distribution, so the defect in the skeletogenous tissue would seem to be the important factor in the development of these muscles. Towards the pelvis the musculature is more nearly normal. Two small pyramidalis are present, extending from the pubis to the rectal sheath, and the caudal portions of the flat muscles are readily defined, but less developed than in a normal fetus at term.

Ventrally between the condyles is a smooth cartilaginous elevation attached to the femur at the site of the patellas trochlea. The inhibition of development here present would seem to be associated with the failure of limb rotation and in particular to the imperfect condition of the quadriceps extensor, which has alike failed to detach the patella and to bring the limb into normal position. The muscle is represented by a small rectus, associated with which are a few fasciculi on each side corresponding to the vastus medialis and lateralis. With the latter the gluteus maximus is continuous. No traces were found of either the vastus intermedius or the subcrureus. The gluteus medius and gluteus minimus are inserted into the greater trochanter on its lateral aspect, into its ventrocephalic surface a fused muscle mass, representing the pyriformis, obturator internus and gemelli, is inserted.

The adductors magnus, longus, and brevis are small; separable at their origins, they insert by a common tendon into a ridge immediately above the medial condyle. The psoas iliacus inserts into the lesser condyle; the pectineus is attached immediately distal to it. No popliteus nor plantaris muscles are present. The gastrocnemius is a very large mass, and no septal division of this mass is found which would reveal an underlying soleus. The former muscle arises primarily from the dorsal aspect of the tibia, however, a few muscular and fibrous prolongations are found attached dorsally to the distal extremity of the femur immediately cephalad to each condyle.

The tibia, like the radius of the upper extremity, is abnormally curved, with the convexity directed ventrad, but not to such a

degree as the radius. The tibial diaphysis is completely ossified, being separated from the epiphyses by intermediate zones of growing cartilage.

There is no fibula.

As a result of the persistent continuity of the patella with the femur, there is no retropatellar extension of the cavity of the knee-joint.

Into the cartilaginous knob, which represents the patella, are inserted a few fibrous bundles from the tendon of the very small rectus femoris.

Here again emphasis should be placed on the fact that the quadriceps extensor muscle is represented chiefly by a rudimentary rectus femoris. The vastus internus and externus possess but a few muscular strands, which arise from the mesial and lateral aspects, respectively, of the greatly shortened femur. The vastus intermedius and subcrureus are absent. We have already noted the fact that the adductors, although present, are very small. Here again the normal stimulus to muscular differentiation and development is either absent entirely or minimal. All the nerves are present. The only absent element which we can discover in the thigh is the diaphysis of the femur. Evidently, then, the teratological evidence indicates that the more rapidly developing skeletal (blastemal-chondrous or osseous) axial zone is the normal stimulus in muscular development, and if it is absent entirely or very much reduced, we find also retarded development in the musculature. This fact further explains the failure of separation and rudimentary condition of the patella as correlated to the inhibited development of the quadriceps extensor.

The flexor muscles of the leg form a larger mass than the extensors. This directs the convexity of the tibial bow ventrad. The large gastrocnemius inserts into the cartilaginous calcaneus. The tibialis posticus and the flexor digitorum longus are fused at their origins, but separable at their insertions. The latter muscle also gives rise to the tendon of the flexor hallucis longus, which has no independent belly.

The tibialis anticus is a large muscle and not only has its normal insertion but distributes the tendons normally belonging to

the extensor longus digitorum. These tendons, however, are small; the latter muscle is absent as well as the three peronei muscles which normally find their origin on the fibula.

In the tarsus the calcaneus, astragalus, cuboid, and navicular are discrete cartilages, but the cuneiform elements form a fused mass.

The metatarsals and phalanges are represented by short, thick cartilage rods. The terminal phalanges have ossification centers at their distal ends. This is comparable to the terminal phalanges of the upper extremity (fig. 8).

The intrinsic muscles of the plantar surface of the foot were not well defined and formed a common muscle mass in which considerable fatty degeneration had taken place.

The condition of club-foot in this monster is readily understood in reference to the extent of development and contraction of the muscles; those belonging to the tibia are well developed, but the fibular muscles are absent except for some of their tendons of insertion which have become amalgamated with the tibial muscles, in consequence the latter group of muscles are practically unopposed in their action. The tibialis anticus manifests its action by inverting, the strong gastrocnemius by extending the foot, resulting in a talipes equinovarus.

In concluding this section I wish again to emphasize the fact brought out in this study, that in the complete absence or rudimentary development of a part of the skeleton, we also find a complete defect or rudimentary development of the related muscles. The converse is also true that an overdevelopment of the skeletal parts is accompanied by a greater degree of development of the related muscles.

B. THE EXTERNAL FORM OF AN ABNORMAL HUMAN EMBRYO OF TWENTY-THREE DAYS

This specimen was given to the writer by Dr. C. J. Nemec, Instructor in Surgery, Creighton University Medical School, January 31, 1917, three hours after miscarriage. The history of the pregnancy is as follows:

The woman, twenty-one years of age, Bohemian, had been married five years. She began menstruating at twelve years of age and invariably suffered from dysmenorrhea. Leucorrhea was always evident two to four days before each period. Her time was irregular; it was not unusual for her to miss a period completely. She had given birth previously to three sickly children, the second of which died two months after birth. The last child was born July 31, 1917. During lactation there was the usual condition of amenorrhea. The nursing child was weaned January 6, 1917. Her first coitus since the birth of her last child was on January 7, 1917. On January 25, she noticed a slight flow, the first since her last conception. This condition of metrorrhagia continued until January 31, 1917. At first she thought this was the reappearance of her normal menstruation. But, on the latter date, a hemorrhagic mass was passed, and then I was called on the case. I saw immediately that the uterine decidua had been passed and I had no difficulty in finding the chorionic sac imbedded in this bloody mass. I put the entire mass in 10 per cent formalin, within one hour after it had been expelled, when I returned to my laboratory.

The chorion was found covered with villi 2 mm. in length. The chorionic sac measured 20 x 19 x 10 mm. I opened it and found the abnormal embryo with its neck extended contained in an amnion with the normal amount of liquor amnii. The chorion was sectioned and was found to be normal, save that it was covered with necrotic syncytium. In this syncytium there was a slight round-cell infiltration. The embryo was unbent in the cervical region obliterating the nape flexure. Especial care was taken to preserve the embryo in the exact position in which it was found. It remained in Zenker's fluid eighteen hours, and excellent preservation was obtained, the surface relief standing out prominently.

Age. No approximate age in days can be computed from the menstrual history, since the woman was in a condition of amenorrhea at the time cohabitation and fertilization occurred. The former took place January 7, and the miscarriage occurred January 31. Undoubtedly, a living ovum was in the outer third of the Fallopian tube at the time of cohabitation. Allowing twenty-four hours until the time that fertilization occurred, the aborted ovum would be approximately twenty-three days old. The embryo is certainly not over one month old, as the external anatomy agrees very closely with the description of embryos between the



Fig. 9 Phocomelus. Skeleton of the lower extremity.

Fig. 10 Twenty-three-day embryo. Chorion (above) and deciduae (below).
A pointer is in the amniotic cavity.

Fig. 11 Twenty-three-day embryo. Ventral view.

Fig. 12 Twenty-three-day embryo. Right lateral view.

Fig. 13 Twenty-three-day embryo. Left lateral view.

twenty-first and the twenty-third days. The embryo stands very close to the embryo *a* of His's normentafeln; embryo 112 of Keibel's collection, normentafeln of Kiebel and Elze, and embryo G 31 of the Anatomical Biological Institute of Berlin. The specimen is younger than the twenty-six-day-old normal embryo described by Mall ('91). In Mall's embryo the eyes are further developed, the liver swelling is more prominent, the branchial arches and clefts are more differentiated, especially the maxillary process of the mandibular arch; the nasal pits are larger, and the limb buds more extended from the body wall. The monster under consideration, however, is older than the 4 mm. embryo described by Bremer, which is estimated approximately at twenty to twenty-one days old. In Bremer's embryo there is no surface marking for the eye and no posterior limbs. The shape, size, and degree of development are midway between Bremer's 4-mm. embryo, aged twenty-one days, and Mall's 7-mm. nape-breech embryo, twenty-six days old, and twenty-three days in all probability is its age. The measurements of the embryo before fixation are as follows:

	<i>mm.</i>
Height of yolk-sac when it projects from umbilicus.....	0.9
Maximal height of yolk-sac.....	2.5
Length of yolk-sac.....	4.0
Length of posterior portion of body, measured from the point of emergence of yolk-sac.....	0.9
From fore-brain to tip of coccyx following curvature.....	13.0
Straight line from boundary between neck and thoracic regions to twelfth thoracic segment (nape-breech).....	40.0
Greater length in a straight line (crown-breech).....	6.0
From vertex to behind the mandibular process.....	0.9
From vertex to behind the heart.....	3.3

External form

It is readily apparent from the photographs that the cervical flexure of the embryo has been abnormally unbent. This is further seen in the sharp groove on the nape separating the neck from the back. There is a very marked degree of curvature noticed in the dorsal, lumbar, and coccygeal segments. If the neck

and head had not unbent, the embryo would form almost a complete circle; the tail would lie in close proximity to the head, if not actually touching it. The body is bent anteriorly and at the same time spirally twisted about its axis so that the head is turned slightly to the right and the pelvic end to the left.

The first, second, third, and fourth arches are clearly defined, especially the nodular ventral end of the mandibular arch (fig. 13). The maxillary processes are perceptible on both sides, not to the extent, however, found in Mall's twenty-six-day old embryo. The second bar is not so bulbous as the first nor the third so prominent as the second. The region of the sinus praecervicalis is distinct, but not so depressed as in Mall's embryo. The fourth arches are barely perceptible on both sides. They lie deep in the groove of the sinus praecervicalis and are covered by the third arches. The clefts begin to show a slight irregularity. Above the branchial region (approximately above the second visceral groove) on both sides there is a small oval depression immediately above the otic vesicle measuring 0.25 mm. in diameter.

The head shows the outline of the brain and the marked elevation over the region of the Gasserian ganglion. The shape of the cerebral hemisphere, the interbrain, midbrain, and afterbrain are plainly recognizable, and the boundaries of the fourth ventricle are sharply defined. From the dorsolateral aspect I was able to define the neuromeres in the lateral walls of the fourth ventricle; these appeared as bilaterally symmetrical transverse folds. The optic vesicles are circular in form on each side and measure 0.3 mm. in diameter. The nasal pits are oval and shallow, but not as large as Mall found them in his embryo. The mouth is a large shallow pentagonal depression bounded above by the nasofrontal process; below and lateral by the nodular mandibular processes on both sides; and lateral, the minute elevations craniad to the mandibular processes, the incipient maxillary processes. It is readily apparent that a line drawn vertically through the ventral ends of the four visceral arches would be approximately straight and would cut the forebrain some distance in front of the optic vesicles. The marked prominence of the forebrain noticed in the phocomelic monster we see is a very characteristic human feature in early embryos.



Fig. 14 Craniorrhachischisis. Ventral view.
Fig. 15 Craniorrhachischisis. Right lateral view.
Fig. 16 Craniorrhachischisis. Skiagraph. Dorsal view.
Fig. 17 Craniorrhachischisis. Skiagraph. Lateral view.

The anlage of the heart projected from the ventral surface of the body as a large nodular swelling; its prolongation on the right side extends forward as the aortic bulb. If the neck was normally bent, the relief of the aortic bulb would extend to the edge of the mandibular arch. The atrial portion of the heart is seen as a protuberance on the lateral wall through the thin wall of the pericardial cavity. The atrial swelling is more marked on the left side and the swelling of the aortic bulb on the right. Caudal to the heart the vitelline vesicle projected from the umbilicus; this vesicle was shrunken and pear-shaped.

The liver swelling is poorly developed and the tail is curved to the left between the cardiac swelling and the body stalk.

The marked coccygeal and pelvic curve is seen from the left as a hook-like process. The tail is conspicuous as is usual in human embryos of this age.

In the posterior region of the trunk four parallel ridges are present; two belong to the axial zones, the medullary and somitic ridges; two belong to the parietal zone, the Wolffian and marginal ridges. There are thirty somites present.

The upper extremities are simply oval mounds; they have not become plate-like as yet. The lower extremities are but ill-defined ridges.

The umbilical cord is large and lies on the left side; a similar condition is found in the embryos described by Mall, Waldeyer, and Janosik, a departure from the usual right-sided position of the cord in human embryos.

C. THE ANOMALIES OF AN ANENĈEPHALIC MONSTER—COMPLETE CRANIORRHACHISCHISIS

The specimen represented in figures 14, 15, 16, and 17, was given to the writer by Dr. J. S. Foote, Professor of Pathology Creighton University Medical School, in 1914. No clinical data were obtainable. The specimen, approximately eight months, old, weighed 1500 grams, and was 20 cm. in length from the breech to base of skull at its dorsal aspect.

The interesting fact in regard to the anencephalic monsters is that they are usually of the female sex; the monster under con-

was represented by mere ridges. The auricular-ventricular valves were quite inadequate and did not function as valves at all. There was evidently marked cardiac incompetency.

In the abdomen the intestines, liver, pancreas, spleen, kidneys, and suprarenals were normal. It has previously been pointed out that there was but one umbilical artery. Upon dissection this proved to belong to the left side. A persistence of but one umbilical artery belonging to the right side was pictured and described by Gillaspie and Henston ('17). In their specimen the uterus was displaced to the left, owing to the fact that the aorta was directly continuous in the median line with the right hypogastric artery. In my specimen a similar arterial condition existed in the pelvis with the difference that the persistent umbilical artery belonged to the left side which in turn caused a displacement of the uterus to the right instead of to the left.

Although the muscular system was dissected and studied, no detailed report will be made here except to state that a sternalis muscle was found on both sides in this specimen, but not in the second monster. The arteries to the limbs were normal as well as the peripheral nervous system. The condition of the skeleton is well depicted in the skiagraph, figures 16 and 17. Note especially absence of the spinal lamina as well as the cranial vault in the dorsal aspect (fig. 16). The base of the skull was accessible to the examining finger. The sella turcica and the anterior and posterior clinoid processes were easily palpated.

The styloid process of the right side is precociously ossified and throws a dark shadow in the skiagraph.

In consequence of the ill-development of the laminae of the cervical vertebrae, there is a lordosis in this region (lateral aspect skiagraph). It is also definitely seen that there is a compensatory kyphosis of the upper thoracic vertebrae, extending the head and allowing it to sink between the shoulders, and giving the fetus an attitude characteristic of many forms of deficient head and spine development. There is also present a marked kyphosis in the lumbar region beginning at the twelfth thoracic vertebra and extending to the first sacral vertebra.

The position of the mandibula and the protrusion of the tongue, which are really an exaggeration of the first act of deglutition, are to be attributed to the imperfect development of the temporal muscles, allowing the depressors to predominate. Of the temporal muscles only the fasciculi arising from the lower part of the fossa are present; these insert upon the coronoid process. They thus are largely representative of the posterior part of the muscle, which is active mainly in retracting the jaw. The large anterior portion, which elevates the jaw, is absent.

All the mandibular depressors are present, with only the small masseter and the internal pterygoid to oppose their action. Accordingly, the typical open mouth of anencephalic monsters is correlated to the defect in the cranial vault and the associated loss of the anterior portion of the temporal muscle.

D. A SECOND ANENCEPHALUS MONSTER—COMPLETE CRANIORRHACHISCHISIS

This specimen was given to the writer in December, 1917, by Dr. T. J. Dwyer, Associate Professor of Surgery, Creighton University Medical School. It was prematurely born at eight months and at first presented by the face. However, because of the overdeveloped shoulder, even more marked than on the forgoing specimen, an obstruction was presented to the descent of the child which called for podalic version. There was a marked condition of hydramnios. Before birth, at six months, the child had been predicted by Dr. Dwyer to be an anencephalos. This condition was suggested by the hydramnios and the exaggerated intensity of the fetal movements which were also irregular and spasmodic in character.

The monster was a female. The condition of craniorrhachischisis was more extensive than in the former specimen. The spina bifida extended through the coccyx. Absolutely no remnants of the calvarium were present. No membranous rudiments were found and only a slight amount of connective tissue covered the base of the skull. Both eyes bulged even more prominently than the left eye of the first specimen because of the greater slant and arrest of development of the supraorbital ridges and plate.

Two arteries and one vein were found in the umbilical cord. No sternalis muscle was found. Outside of these differences the description of the first specimen holds for the monster under consideration; but the abnormalities of the heart are if anything more extensive.

Ahlfield ('80) assigns as the direct and immediate cause of anencephalic monsters, hydrocephalus. If the serum accumulates early within the ventricles, the brain and its covering are ruptured at about the fourth week of embryonic life, they atrophy and disappear and the result is anencephalous. The accumulation of serum may make it impossible for the bony case of the brain to enclose the cranial cavity, causing thus varying defects of the skull through which the membranes and their contents protrude. If the serous effusion affects the spinal region as well as the cranial cavity before closure of the neural tube, which is thus prevented, there is an associated spina bifida resulting therefore in the condition of *craniorrhachischisis*.

The primary cause of hydrocephalus can only be surmised. Many have made a vague reference to the already overworked amniotic bands. It is interesting to note that Morgan has experimentally produced spina bifida in the tadpole of frogs by subjecting the eggs to a 0.6 per cent solution of common salt. This retards development and results in posterior spina bifida. In regard to the underlying cause of anencephalic monsters with spina bifida, Mall ('10) concludes: "It is no longer necessary for us to seek mechanical obstructions which may compress the umbilical cord, such as amniotic bands, for it is now clear that the impairment of nutrition which naturally follows faulty implantation or the various poisons which may be in a diseased uterus, can do the whole mischief."

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Resumido por la autora, Mary Drusilla Flather.

La irrigación sanguínea de las áreas de Langerhans; estudio comparativo del páncreas de los vertebrados.

El presente trabajo es el primero de una serie de estudios comparativos sobre la irrigación sanguínea especializada en las áreas de Langerhans. La introducción contiene un corto resumen de los trabajos ya verificados en el campo general sobre el origen y función de las áreas insulares y su estructura histológica. La autora hace un estudio comparativo de la disposición de las células y vasos sanguíneos en las áreas insulares del aligador, oposum, caballo, racoon (*Procyon lotor*), badger (*Taxidea taxus*), skunk (*Mephitis putida*), conejo y conejillo de Indias. Los resultados obtenidos llevan a la conclusión de que, mientras que las áreas vasculares son en extremo variables, incluso en el mismo individuo, hay ciertos rasgos distintivos—forma, tamaño, red sinusoidea, etc.,—que caracterizan a los islotes de las diferentes especies de vertebrados. El trabajo está ilustrado con ocho figuras de los ejemplares examinados, dibujadas con la cámara clara. La técnica empleada en la obtención de las preparaciones se describe en el texto.

Translation by José F. Nonidez
Columbia University

THE BLOOD SUPPLY OF THE AREAS OF LANGERHANS, A COMPARATIVE STUDY FROM THE PANCREAS OF VERTEBRATES. (PRELIMINARY PAPER.)

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EIGHT FIGURES

INTRODUCTION

Since 1895, when the islets of Langerhans were declared by L. A. Shaeffer to be endocrinous glands, secreting a substance capable of modifying the metabolism of carbohydrates in the tissues, the study of these organs has been provocative of deep interest and much controversy. It is my purpose in this paper to present a purely comparative study of the specialized blood supply in the islets. Therefore, in my consideration of the work already accomplished in the general field, I shall mention only those facts which are necessary for an adequate comprehension of my problem. A detailed summary of the literature up to 1906 is given by Laguesse in *La Revue Generale d'Histologie*, vol. 2, 1906-1908. Two important contributions since then are the papers of Lydia M. Dewitt in *The Journal of Experimental Medicine*, 1906, vol. 8, and of R. R. Bensley in *The American Journal of Anatomy*, 1911-1912, vol. 12.

It is generally conceded that islets are groups of internally secreting glands embedded in the pancreatic tissue of all species of vertebrates. Their origin is still a matter of controversy, although the careful work of Dewitt and of Bensley seems to prove conclusively that the islets and acini arise from common anlagen, later becoming differentiated and incapable of transformation one into the other. The cells, varying in form and structure, are always arranged in cords or masses separated by

large anastomosing blood-vessels. The nature of this vascular network is analogous to that found in other endocrinous glands, especially the thyroid and suprarenals. According to Jordan and Ferguson, certain arterial branches enter the islets and form a plexus of large capillaries from which the blood is drained through the venous system. Dewitt, however, claims that the sinusoids communicate intimately with an interacinar capillary plexus and with larger vessels of venous origin only, basing her theory upon her inability to find near the islets any of the characteristic arterial endothelium.

The islets vary in size and shape according to their vascular content and according to whether they are singular or compound. Harris and Gow find three distinct types of islets—those which are lymphoid in appearance with many small, deeply stained nuclei in a syncytial mass of tissue; those having distinct cell outlines, and those consisting of compound cell groups divided into smaller areas by strands of connective tissue. It is probable that the first two types represent physiological differentiation only. Usually the cells toward the periphery of the islet are massed, while those in the center are arranged in irregular cords one or two cells in depth resting directly on the walls of the capillaries. As a rule, a connective-tissue sheath surrounds the islet, sometimes penetrating within, and frequently failing to separate the acinous and islet cells completely. It is often difficult to distinguish the islet from the peri-insular zone, but in general the islet cells may be recognized by their polymorphism, their slight colorability, their small size, and the large chromatin content of their nuclei. In addition to the rich blood supply there is also in the islet a plexus of nerve fibers, which was shown by Pensa to pass along the blood-vessels and in between the cells.

TECHNIQUE

In my own investigations on the histology of the islet cells I am much indebted to Dr. Frederick M. Allen for supplying me with the greater part of the material which I have used. The specimens of alligator, opossum, horse, coon, badger, and skunk

pancreas which I obtained from him had been fixed in either chrome sublimate or Zenker's solution and embedded in paraffin. From these blocks I cut sections 3 to 5 μ thick, and stained them all in Mallory's connective-tissue stain before mounting them for observation. Fresh material was taken from a guinea-pig and a rabbit. Following Bensley's directions, I employed all four of his methods of fixation, and used the neutral gentian stain. The most successful results were from the Zenker bichromate sublimate fixation. Both neutral gentian and Mallory's connective-tissue stain proved excellent in the differentiation of the islets and the surrounding tissue. The drawings were made with a camera lucida, using a Zeiss oil-immersion lens and no. 4 ocular, resulting in a total magnification of 1250.

THE BLOOD SUPPLY OF THE AREAS OF LANGERHANS

A cursory glance at the accompanying figures shows how varied is the arrangement of the vascular areas in the islets of Langerhans. To a certain extent there may be variation even in the islets from the same individual, but I shall endeavor to show that there are certain distinctive features which characterize islets in the different species of vertebrates.

In the alligator, figure 1, the islets are noticeably large, compound, and syncytial in appearance, with a very appreciable granular content due to great physiological activity. This latter feature may be due merely to the youth of the specimen. With the physiological vascular injection produced by congestion it is easy to see how the distended blood-vessels surround the area, rarely penetrating within it, except in the connective-tissue sheaths, and forming almost a complete barrier between the islet and acinous cells. There is no differentiation between the peripheral cells and those of the interior of the islet, and no indication of a capillary network. In ten islets from the same individual there was no appreciable variation except in size. Many of the islets were larger than the one represented here.

The islet from the opossum, figure 2, presents a quite different appearance with distinct cell divisions, a sinusoidal arrangement

of blood-vessels in the interior of the area, no encircling vessels, and no definite capsule or sheath to separate the islet from the acinous cells. A peculiarity of the cell arrangement not found in any other pancreas observed is the radial grouping around capillaries, which is most suggestive of the radial form of acinous cells about their lumina. These essential features were found in ten islets from the same pancreas.

The islet from the horse, figure 3, is definitely ovoid in shape, and is surrounded by a frame of blood-vessels and connective tissue, which in places penetrate within the area. The cells are clearly outlined, but show no differentiation between the central and peripheral grouping in any of the ten islets studied.

The distinctive feature of the raccoon islet, figure 4, is the compound form and lobular appearance. The smaller masses are separated by a network of blood-vessels and connective tissue which also lies between the acinous cells and the large islet area. Another characteristic is the extensive penetration of the lobules by the capillaries. The cell outlines are fairly distinct, but again there is no differentiation, even where the blood-vessels have invaded the central mass. There was no variation worthy of note in ten islets from the same pancreas.

As might be expected from their close relationship, the skunk and badger, figures 5 and 6, have islets with many similarities of structure. In both, the blood-vessels only partially separate the acinous from the islet cells. There is a definite sinusoidal network running through the central mass. This makes it possible for nearly every cell to come in contact with the capillaries, a feature which should greatly facilitate the circulation of the secretion. Figure 6 shows a large accumulation of interlobular connective tissue at one side where the area of the islet reaches the periphery of the lobule. From a study of ten islets from

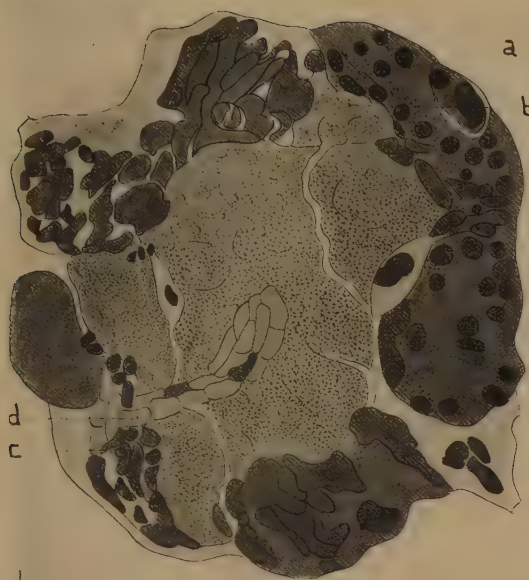
Fig. 1 Island of Langerhans from young alligator. Aq. chrome sublimate Mallory. $\times 417$.

Fig. 2 Island of Langerhans from opossum. Zenker Mallory. $\times 417$.

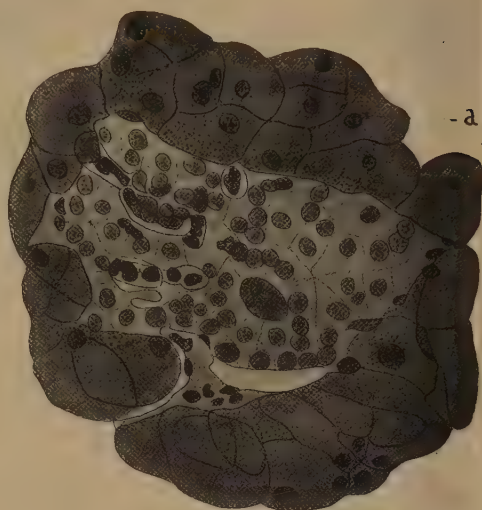
Fig. 3 Island of Langerhans from horse. Aq. chrome sublimate Mallory. $\times 417$.

Fig. 4 Island of Langerhans from racoon. Zenker Mallory. $\times 417$.

a, acinous cells; b, islet cells; c, blood vessels; d, connective tissue.



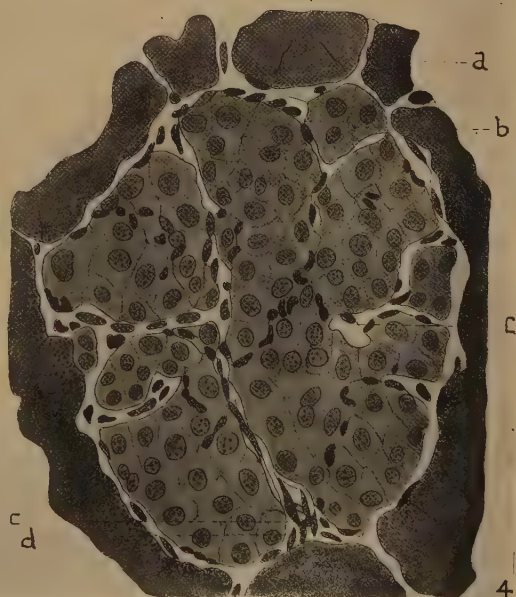
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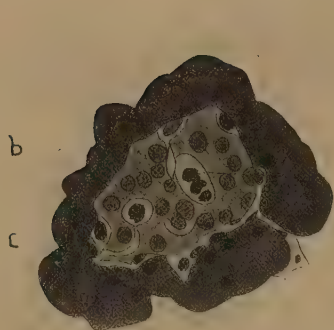
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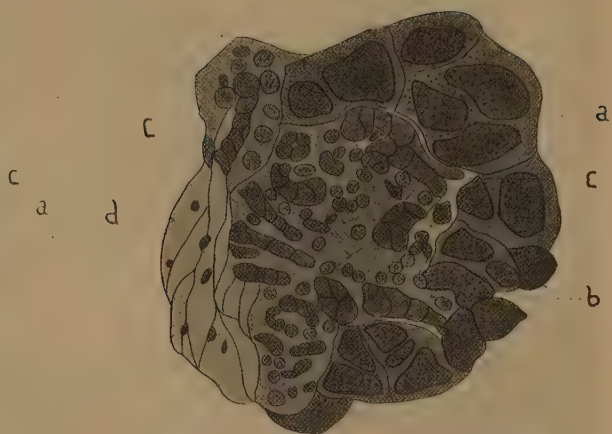
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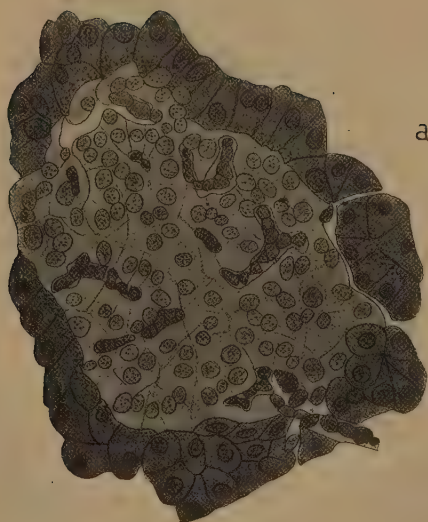
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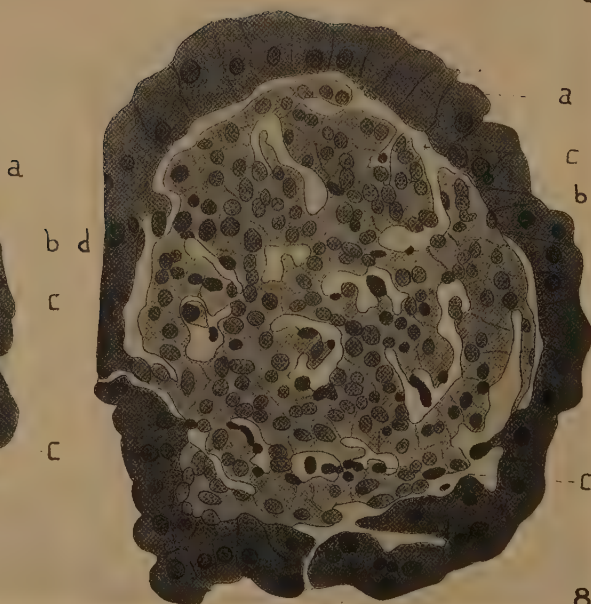
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each individual it was concluded that the islet areas were smaller in the skunk than in the badger pancreas.

In the islet of the rabbit, figure 7, the line of demarcation between the islet and acinous cells is difficult to define because the former frequently extend into the acinous area and there is no circle of blood-vessels or connective-tissue sheath to mark the division. The blood supply is sinusoidal in nature. In this form there is a slight indication of cord-like cell grouping and a massing of cells with larger nuclei toward the periphery. Unlike Dewitt, I found in the ten islets examined no radial arrangement similar to that which was observed in the opossum.

The guinea-pig islet, figure 8, shows a fairly regular contour, a connective-tissue sheath separating the islet and acinous areas, and an irregular network of insular cells frequently but one cell in depth surrounding the large and abundant sinusoids. After careful observation of islets from two individuals and with due allowance for faulty fixation, I decided that these islet areas were the most sponge-like of any that I have examined.

From the islets which I have described I feel that there is an arrangement of the cells and blood-vessels which may be regarded as characteristic of a species. Within certain limits there may be variation in size and in abundance of capillaries with a consequent rearrangement of the islet cells. However, I believe that the special features can be proved peculiar to the species. I realize that the proof is inadequate as yet owing to the fact that with one exception I have studied islets from only one individual of a species. It is my intention to continue the investigation with many more species and more individuals of the species. I am greatly indebted to Dr. David H. Tennent for his helpful supervision of the work.

Fig. 5 Island of Langerhans from skunk. Chrome sublimate Mallory. $\times$ 417.

Fig. 6 Island of Langerhans from badger. Chrome sublimate Mallory. $\times$ 417.

Fig. 7 Island of Langerhans from rabbit. Zenker neutral gentian. $\times$ 417.

Fig. 8 Island of Langerhans from guinea-pig. Zenker neutral gentian. $\times$ 417.

a, acinous cells; *b*, islet cells; *c*, blood vessels; *d*, interlobular connective tissue.

Resumido por la autora, Inez Whipple Wilder.

Una anomalía de la circulación de la porta en el gato.

En un gato macho de gran tamaño y aproximadamente de un año de edad, un espacioso canal sanguíneo colateral, formado por la anastomosis de los tributarios de la porta con la vena frénica izquierda, hacía posible el paso directo de la sangre desde dichos tributarios a la vena postcava, evitando de este modo el trayecto normal a través del hígado, si bien este trayecto estaba abierto. Con esta anomalía estaban asociados: un aumento de tamaño de los riñones y una disposición irritable en extremo, por parte del animal.

Translation by José F. Nonidez
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AN ANOMALY IN THE PORTAL CIRCULATION OF THE CAT

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FOUR FIGURES

While injecting the circulatory system of a cat recently, I noticed that the injection of the systemic veins through the right femoral resulted in nearly filling the hepatic portal tributaries, so that when I came to make the usual yellow injection of the portal system through one of the mesenteric veins, I found these already filled with the blue venous injection mass.

Upon dissecting this specimen I found that the hepatic portal vein was small, while there was a very large collateral connection between the hepatic portal system and the postcava. A comparison of this aberrant condition (figs. 1 and 2) with the normal condition (figs. 3 and 4) makes it evident that this collateral is formed by the anastomosis of the coronary veins of the stomach with the left phrenic vein, so that the collateral vein thus formed extends along the lesser curvature of the stomach, and enters the postcava at the level of the diaphragm. A voluminous anastomosis of the gastrosplenic vein with this collateral furnishes a very direct channel into the postcava from the whole system of mesenteric tributaries, as well as from the gastrosplenic vein itself. Near the junction of the collateral with the main portal vein; the collateral is joined by the combined pancreaticoduodenal and gastro-epiploic veins, thus completing the direct connection of all of the portal tributaries with the postcava through the coronary collateral. The blue injection mass had thus backed into the portal system directly from the postcava and had entered not only all of the portal tributaries, but the portal vein itself and all of its branches to the various lobes of the liver.

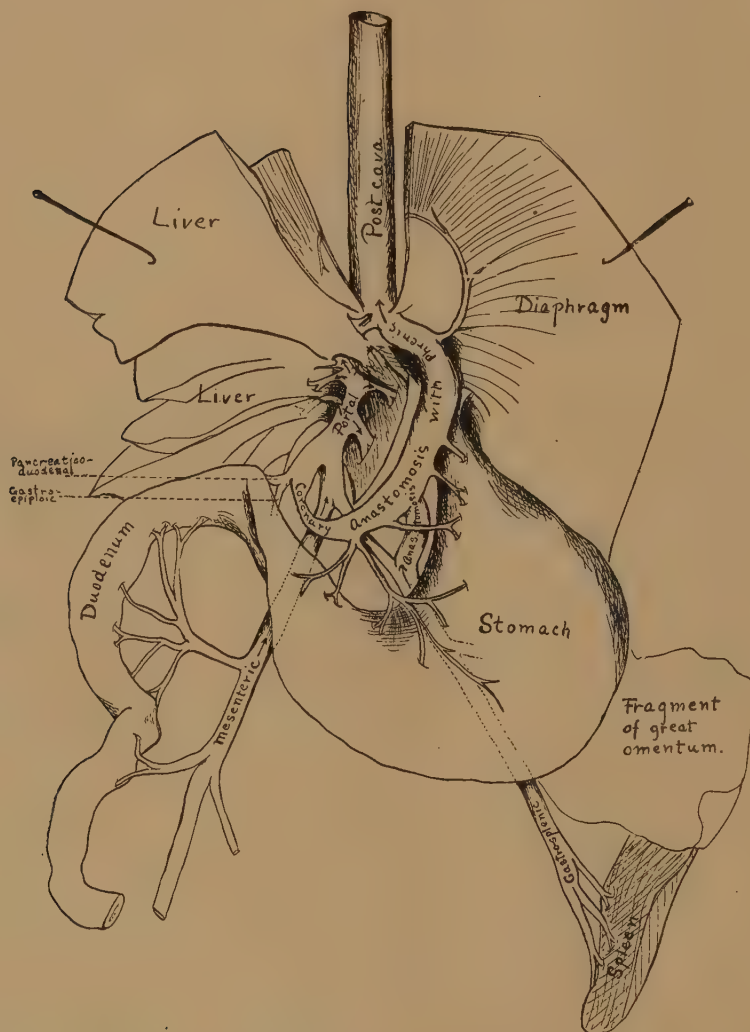


Fig. 1 Ventral view of dissection of anomalous cat showing the anastomosis of the coronary and gastrosplenic veins with the left phrenic, resulting in the formation of a direct collateral drainage from the portal system into the postcava. The diaphragm is represented as slit from the midventral line to the postcava, the liver is lifted, and both liver and diaphragm are drawn anteriorly to display the relationships of the blood-vessels.

It seems probable from the large size of the collateral vein as compared with the unusually small size of the main portal vein, that a large proportion of the blood had habitually escaped its normal course through the liver capillaries, and had entered directly into the main circulation, carrying with it continually an excess of nutritive material and unconverted nitrogenous wastes.

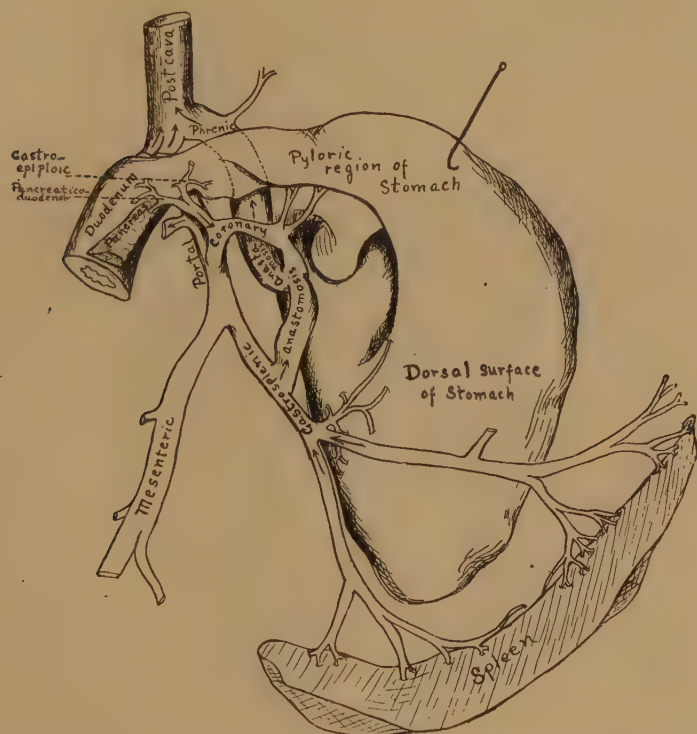


Fig. 2 Details of the connections of the portal system with the collateral channel in the anomalous individual, shown by lifting the pyloric end of the stomach and carrying it anteriorly and to the left.

It could scarcely be imagined that such a condition would not be accompanied by other abnormalities if not by actual pathological conditions. There was, however, nothing unusual in the appearance of the freshly killed specimen, which was a rather large male, well developed, but not unusually fat. Unfortu-

nately, the abnormal condition of the circulatory system was not discovered in time to make any histological study of liver or kidneys. In fact, no examination of these organs was made while the specimen was fresh, and as the preserving fluid had not well penetrated the anterior abdominal organs, it was impossible to

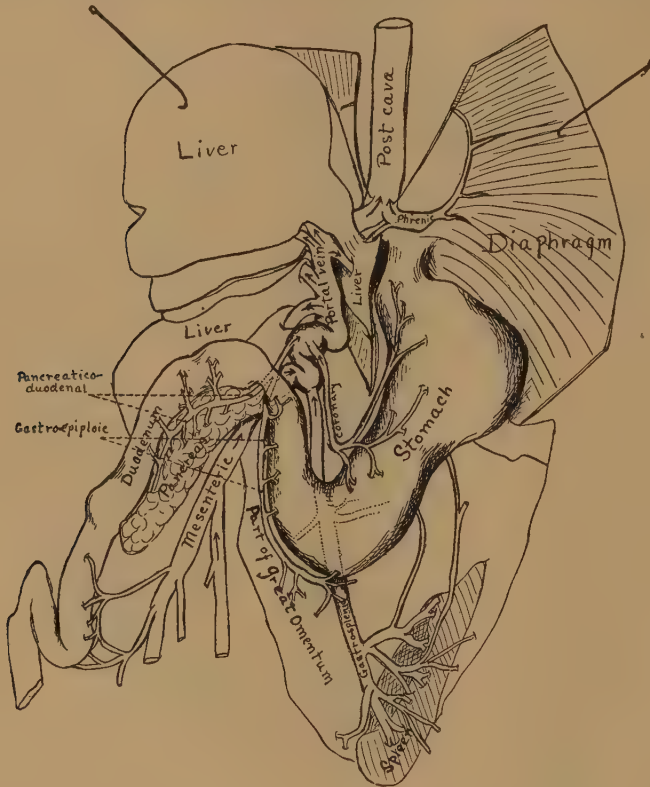


Fig. 3 Ventral view of dissection of normal cat seen from a point of view similar to that of figure 1.

determine whether the somewhat shrunken and flabby appearance of the liver was due to faulty preservation or was the condition of the organ during life.

On the other hand, as might be expected from the inevitable increase in the amount of work devolving upon the kidneys as a

result of the interference in the normal functions of the liver, the kidneys were undoubtedly enlarged. This fact is shown by the accompanying tabulation of measurements of kidney and total body lengths made upon the abnormal individual and upon ten apparently normal individuals taken at random from specimens

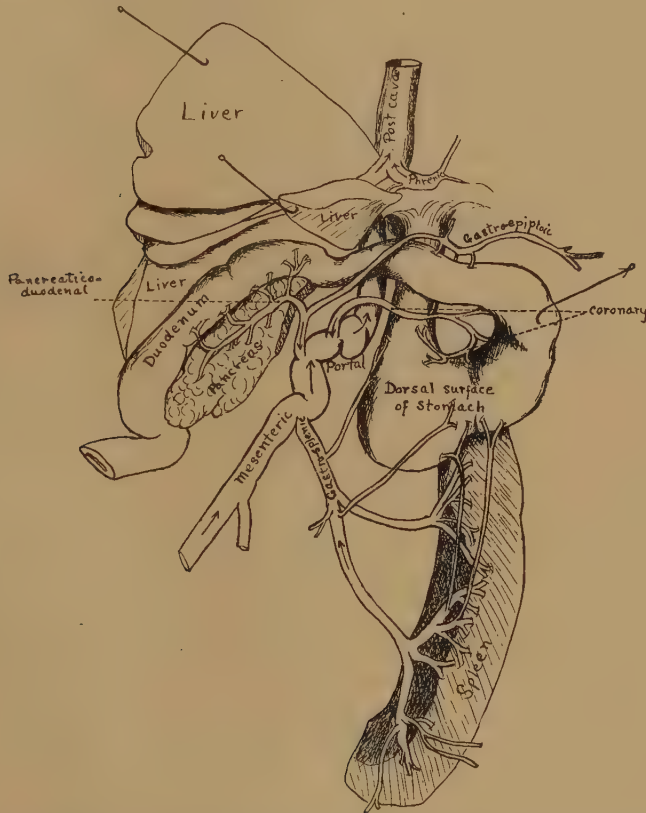


Fig. 4 Details of relationships of portal tributaries in the normal individual seen from a point of view similar to that of the anomalous individual shown in figure 2.

in the laboratory similarly injected and preserved by the same method as the abnormal one. The total length was measured from the tip of the nose to the posterior end of the ischiadic symphysis with the specimen lying upon its back upon the table, and

the head held back to approximately the same degree. In the very nature of the case, however, these total-length measurements have only an approximate degree of accuracy.

TABULATION OF MEASUREMENTS

SEX	TOTAL LENGTH	AVERAGE LENGTH OF KIDNEYS	PROPORTIONATE LENGTH OF KIDNEYS	REMARKS
Normal individuals				
	<i>mm.</i>	<i>mm.</i>	<i>per cent</i>	
Female.....	430	31.5	7.3	
Male.....	490	43.0	8.8	
Female.....	470	40.0	8.5	
Female.....	415	30.0	7.2	
Female.....	475	38.5	8.1	
Female.....	480	37.0	7.7	
Female.....	450	43.0	9.6	Gravid (advanced)
Female.....	430	37.5	8.7	
Male.....	480	49.0	10.2	
Female.....	450	36.0	8.0	Gravid (advanced)
Average.....	456	38.5	8.43	
Abnormal individual				
Male.....	500	60.0	12.0	

It will be noted that although there is a considerable range of variation in both the actual and the proportionate length of kidneys of the normal individuals measured, in none of these does either the actual or the proportionate length equal that of the abnormal individual.

Inquiries were made to determine, if possible, whether there had been anything abnormal in the behavior of the cat or any indications in its history of a pathological condition. It was learned that the cat was about a year old and had always been peculiarly active and irritable. Even as a kitten it had never tolerated petting, and, to quote the informant, a member of the family of the donor, "it was the strangest acting cat" he had ever seen. It was at first denied, however, that the cat had ever been patho-

logical, but the admission was finally made that it had had a 'fit' a short time before it had been donated to the laboratory, and that this fact, together with the increasing excitability of the animal, had led to its being donated to the laboratory. This account points rather significantly to an inability of the kidneys to cope fully with the extra work devolving upon them as a result of the interference with the full function of the liver.

This case would seem also to have some embryological significance, since, as pointed out by Huntington and McClure ('07) in referring to conclusions based upon the dissection of 605 cats by Darrach ('07) although "the average individual assumes the venous and lymphatic type considered normal for the species," by the well-known process of selection and continued development of certain embryonic pathways while others undergo degeneration, it has been found possible to "interpret all of the observed adult variants as examples of atypical persistence of early channels normally destined to disappear in the course of further development, but capable, by continued and unusual growth, of affording all of the variations of the adult venous system observed in the cat."

So far as I know, an anomaly of this particular type has not before been reported. Undoubtedly, however, when the full report of the embryological evidence collected by Huntington and McClure is published, this case will fall into its proper place as one of the variants due to the atypical persistence of embryonic channels.

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Resumido por el autor, Harrison R. Hunt.

Anomalias vasculares en un gato doméstico (*Felis domestica*).

Las anomalias vasculares descritas a continuación han sido observadas en un gato adulto. Las del sistema venoso eran: Una postcava izquierda, venas renales dobles en cada lado, un orificio en la vena iliolumbar izquierda a través del cual pasa la arteria correspondiente, vena espermática izquierda ramificada desde la postcava y la misma vena del lado derecho ramificada desde una de las venas renales derechas. El uréter estaba rodeando a la postcava. En el sistema arterial, el arco aórtico estaba situado en el lado derecho y la arteria innominada en el izquierdo; desde esta última se ramificaba la arteria carótida común y la subclavia izquierda, mientras que la subclavia derecha arrancaba de la base del cayado de la aorta.

Translation by José F. Nonidez
Columbia University

VASCULAR ABNORMALITIES IN A DOMESTIC CAT (FELIS DOMESTICA)

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ONE FIGURE

Recently the writer dissected a male cat which presented so many interesting vascular anomalies that publication of the facts seemed justified. The accompanying figure is a semidiagrammatic representation of the main blood-vessels of this animal as seen from the ventral side.

Posterior to the superior mesenteric artery (12) the postcava (9) was situated at the left of the aorta (10).¹ The left ureter (28) looped around the postcava in the manner shown in the figure. The position of the spermatic veins was the reverse of the normal position, the left spermatic (23) branching from the postcava (9), while the right (20) emptied into the posterior right renal vein (17).

Other observers have reported similar abnormalities in the cat. Darrach ('67) has described three cases in which the relations of the postcava, ureter, and sex veins were practically the same as in this individual. McClure ('00) figures (fig. 3) a case in which each ureter looped around the persistent postcardinal vein, as the left ureter passed around the postcava in the accompanying figure. Hochstetter ('93) mentions one cat in which the postcava lay at the left of the aorta posterior to the superior mesenteric artery, and a second case (having two persistent postcardinal veins) in which each ureter looped around a postcardinal.

Two renal veins (17 and 19) drained each kidney (14). Double renal veins were observed by McClure also ('00) in the cat.

¹ Unfortunately, my records do not show whether the superior mesenteric and coeliac arteries were on the right or the left side of the postcava.

The left iliolumbar artery (26) passed dorsally through a foramen in the left iliolumbar vein. Such venous foramina have been reported by several other observers (Darrach, '07; McClure, '00; Treadwell, '96; Weyssse, '03; Smallwood, '06).

The explanation for these anomalies in the venous system must be sought in embryology. Most of the distal portion of the left postcardinal vein in the cat embryo normally degenerates anteriorly to the level at which the left spermatic vein branches off (Hochstetter, '93, '06). On the other hand, the right postcardinal vein persists, becoming part of the postcava. Normally, the right spermatic vein permanently maintains its embryonic connection with the right postcardinal. But apparently in this animal the postrenal part of the left postcardinal, instead of the right, persisted as part of the postcava. Consequently the left spermatic vein retained its embryonic relation with the left postcardinal.

Probably the right postcardinal of this animal degenerated anteriorly as far as the right spermatic vein, so that the latter became a branch of the right renal.

Hochstetter's ('93, '06) observations on the development of the cat's veins show clearly why the left ureter encircled the postcava in this animal. In the cat embryo each ureter is surrounded, at a certain stage, by a venous island, consisting dorsally of a supracardinal vein, ventrally of the postcardinal (Prentiss, '15, fig. 274). In the cat, according to Hochstetter, and in other mammals only the dorsal, or supracardinal, limb of this island persists as a part of the postcava. In this particular cat, and in similar cases which have been reported, doubtless the supracardinal limb of the island degenerated, and the postcardinal limb survived as a part of the postcava, causing the ureter to pass around the dorsal side of the postcava (Metcalf, '18).

Probably the most infrequent abnormality in this cat was the position of the aortic arch. Normally it lies on the left side of the animal, but in this case it was on the right side. The left subclavian artery (4), instead of connecting directly as usual with the aortic arch, came from the distal end of the unusually short innominate artery. The right subclavian (6) branched

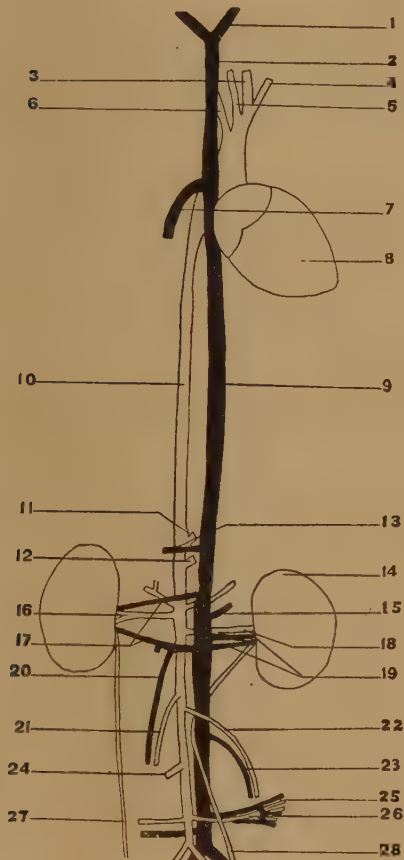


Fig. 1 Ventral view of the arterial and venous systems. The hepatic veins have been omitted. 1, left innominate vein; 2, precava; 3, left common carotid artery; 4, left subclavian artery; 5, right common carotid artery; 6, right subclavian artery; 7, azygos vein; 8, heart; 9, postcava; 10, aorta; 11, celiac axis; 12, superior mesenteric artery; 13, right adrenolumbar vein; 14, left kidney; 15, left adrenolumbar vein; 16, right renal artery; 17, right renal veins; 18, left renal artery; 19, left renal veins; 20, right spermatic vein; 21, right spermatic artery; 22, left spermatic artery; 23, left spermatic vein; 24, inferior mesenteric artery; 25, iliolumbar vein; 26, iliolumbar artery; 27, right ureter; 28, left ureter.

from the aortic arch at the base of the innominate. In man also the aortic arch occasionally occurs on the right side (Cunningham, '09).

Mammalian embryology furnishes an explanation for the anomalous position of this arch. In the normal development of mammals the right fourth arch degenerates, leaving the left fourth arch to carry the blood from the heart. Probably the left fourth arch of this animal degenerated, as in birds, leaving the arch on the right side as the permanent blood channel.

It would be interesting to know whether these vascular abnormalities were due to hereditary or environmental influences, to one cause or to a chance combination of several independent causes. All these anomalies were not produced by the disappearance or unusual modification of the normal activity of one Mendelian factor, for all these abnormalities seldom occur together in one animal. Nevertheless, the normal and abnormal conditions of these vessels may possibly be Mendelian characters, the occurrence of so many abnormalities in one animal being a very unusual chance combination of characters.

Or, possibly, the developing embryo was subjected to unusual environmental conditions, such as abnormal amounts of certain substances in the mother's blood. These conditions may have slightly modified the development of the body as a whole, but produced most pronounced abnormalities in the blood system. Investigations in genetics or experimental morphology might furnish a satisfactory explanation.

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Resumido por el autor, Ezra Allen.

Degeneración en el testículo de la rata albina a consecuencia de una dieta deficiente en la vitamina soluble en el agua, con una comparación de una degeneración semejante en ratas tratadas de un modo diferente y una consideración sobre el tejido de Sertoli.

Las ratas albinas sometidas por Osborne y Mendel a una dieta deficiente en la vitamina soluble en el agua son estériles. El exámen de los testículos ha demostrado la degeneración completa de las células germinales. Tan solo persisten en los túbulos las células de Sertoli, si bien sus núcleos aparecen muy contráidos. El tejido intersticial estaba hipertrofiado. Estos caracteres son los mismos que se encuentran en los testículos de otros mamíferos sometidos a la acción de los rayos X. Una degeneración semejante ha sido observada también en algunas ratas alcoholizadas por MacDowell, degeneración que se presentaba también, aunque en menor grado, en los hermanos de dichas ratas que no fueron sometidos a la acción del alcohol. Bajo estas condiciones el tejido de Sertoli revela una estructura sincicial que el autor del presente trabajo considera como el estado normal, como demuestra el material bien fijado.

Translation by José F. Nonidez
Columbia University

DEGENERATION IN THE ALBINO RAT TESTIS DUE TO A DIET DEFICIENT IN THE WATER-SOLUBLE VITAMINE, WITH A COMPARISON OF SIMILAR DE- GENERATION IN RATS DIFFERENTLY TREATED, AND A CONSIDERATION OF THE SERTOLI TISSUE

EZRA ALLEN

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SEVENTEEN FIGURES

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INTRODUCTION

This paper deals with a similar type of degeneration found in the testes of two groups of albino rats which had been subjected to very different treatment. The histological and cytological

conditions revealed upon examination are of sufficient interest for description not only for the light they throw upon certain problems connected with the Sertoli tissue, but also because they indicate the value of further experiments along the lines of their causes.

THE MATERIAL

I am indebted to Professors Osborne and Mendel for one lot of rats and to Dr. E. C. MacDowell, of the Carnegie Station for Experimental Evolution at Cold Spring Harbor, for the other. There were four in the first lot and ten in the second group. For convenience I will refer to the two lots as the Osborne and Mendel and the MacDowell rats.

The first lot were chosen at random from a larger group which had been subjected to a diet deficient in the water-soluble vitamine. All of these rats, both male and female, had proved sterile. Aside from their sterility they were very well developed. The data of especial interest as to their development will be found in table 1.

The MacDowell rats came from a lot which have been referred to in a paper published by MacDowell and Vicari ('17) dealing with reduction in fertility among rats subjected to alcohol. Five of the alcoholized rats, from five different litters, and their normal brothers were sent to me. The alcoholics had been made drunk daily beginning at twenty-eight days of age. Each treatment was continued long enough to produce not only

TABLE 1

Showing the body measurements, comparison of weight of testes with standard on body length, and age at death of the Osborne and Mendel rats

NUMBER	WEIGHT	LENGTH OF BODY + TAIL	LENGTH OF BODY	WEIGHT OF TESTES	STANDARD WEIGHT OF TESTES ON BODY LENGTH	AGE AT DEATH
	<i>grams</i>	<i>mm.</i>	<i>mm.</i>	<i>grams</i>	<i>grams</i>	<i>months</i>
3554	316	400	230	0.975	2.278	24
3610	325	438	237	0.690	2.926	22
3756	348	397	218	0.851	2.525	20
3896	288	414	222	1.334	2.609	18

inability to stand, but frequently to render the rats absolutely motionless. This meant long treatments, since as they became habituated they could stand larger and larger amounts before they succumbed.

All ten had been subjected to a special diet for fifteen days, beginning when they were seventy days of age. It consisted solely of white bread soaked in fresh milk. The rats were allowed to eat of it for thirty minutes the first two days, for fifteen minutes the next five days, and for five to ten minutes the next eight days.

Data for these rats, corresponding to those for the Osborne and Mendel lot, are to be found in table 2.

TECHNIQUE

The two sets of rats were treated substantially alike, although the MacDowell rats were killed in May, 1917, and the Osborne and Mendel in April, 1918. After etherization they were measured and weighed. The testes were then removed and, with the exception of Osborne and Mendel's nos. 3554 and 3756, dropped into the fixative, B-15 at about 38°C., after being cut into small pieces by scissors. The fixative had been weighed previously, so that a second weighing with the testes in it gave data for determining their weight. The two rats, nos. 3554 and 3756, were injected by the fixative after washing out the blood-vessels with Locke's solution. For the sake of uniformity in weights, the testes of all the Osborne and Mendel rats were weighed after fixation.

The fixative was replaced with 70 per cent alcohol by the drop method, the picric acid washed out with the help of lithium carbonate in 70 per cent alcohol, and dehydration completed in anilin oil. Clearing was by oil of wintergreen. Both oils were added by the drop method. Infiltration by paraffin was brought about very gradually. Details of this treatment are described in my paper on technique (Allen, '16). Sections were cut at 7 μ and 10 μ and stained with iron haematoxylin and acid fuchsin or orange G.

OBSERVATIONS UPON THE OSBORNE AND MENDEL RATS

In the case of all four rats the tunica albuginea was found to have an excess of a clear, serum-like fluid, which flowed out as soon as the tunic was ruptured. The solid part of the testes was much shrunk. The fluid had distended the organs so that they looked quite normal before the tunic was ruptured.

Upon examination of the sections certain conditions common to all four were revealed. Some minor variations in the different individuals were present. The common conditions will be described first. These may be enumerated as 1) no mature spermatozoa; 2) almost no normal germ cells in any stage; 3) generally speaking, an absence of spermatogonia, spermatocytes, and spermatids; 4) the Sertoli tissue the most prominent of any tissue in the tubules, and 5) an increased quantity of interstitial tissue as compared with the normal.

An idea of the general appearance of the tubules and the interstitial tissue may be had by examining figures 2 and 9. It will be noted that the germinative epithelium is almost entirely lacking or very abnormal, the latter state due to the presence of numerous cavities. Figures 5 and 6 show these conditions better. Sometimes the tubules are apparently solid, no lumen appearing, and the cavities just referred to either lacking entirely or, if present, very small (figs. 1 and 8). In either case the contents of the tubules are composed chiefly of Sertoli nuclei and their syncytial cytoplasm. Since this tissue is the most prominent, it will be described first, after a brief consideration of its normal structure.

Normal Sertoli tissue

In normal tubules the syncytium is difficult to see, on account of the closely packed germ cells, but under favorable conditions in the section very small areas may be discerned. Close to the Sertoli nuclei the cytoplasm is clearly observable, but no cytoplasmic walls are to be seen. In character the syncytial cytoplasm in both the normal and in these degenerate conditions resembles the substance which fills the tubules in the early

stages of their development. See my figures 2 to 8, Allen (18). In the adult it is best seen when the groups of spermatozoa have nearly matured and are migrating toward the lumen from the immediate neighborhood of the Sertoli nuclei along the basement membrane. These groups of spermatozoa then seem to have dragged with them some of the Sertoli cytoplasm, which under favorable conditions may be seen extending nearly across the entire width of the germinal epithelium. It is distinguished by its coarse nature and its heavy staining character. Even then no cytoplasmic wall is visible. This appearance is shown in figure 14.

The Sertoli nuclei are nearly always in close relationship with the basement membrane (fig. 14). They may be distinguished by the nucleolus, which is well illustrated in figures 14 to 17, and by the less active reaction of the nuclear plasma to haematoxylin as compared with that of the spermatogonia.

The Sertoli tissue in the degenerate tubules

To return now to the degenerate tubules under consideration: the nuclei are irregular in outline, due to their membranes having wrinkled and formed grooves of various sizes (figs. 11 to 13). Several small bodies staining like chromatin are present. Two, twin-like, are the most prominent. These are shown in figures 11 and 13. In the normal Sertoli nuclei these bodies are very unequal in size, as shown in figures 14 to 17. Intermediate stages have been traced between the normal and degenerate condition with respect to this duplex body, so we may be confident that such nuclei as shown in figures 11 and 13 are Sertoli cell nuclei.

In such a stage of degeneration as shown in figure 6, these nuclei are very numerous and are scattered throughout the tubule. In the more advanced stage, as illustrated in figure 5, they are confined to the periphery, and are reduced in size and in number. A rough idea of this diminution may be obtained by a comparison of the tubules photographed for figures 5 and 6. They are from the same section, which was cut at 7μ .

In the tubule shown in figure 6, fifty-one nuclei are to be counted in the section; in the one shown in figure 5, thirteen are present. In neither case are any other kinds of cells to be seen within the tubules. The true germ cells have completely disappeared.

The cytoplasmic substance stains with orange G and with acid fuchsin more lightly than the same substance in the young tubule, but seems very similar in structure. It may be described as a loose meshwork, the substratum of which seems to be minute, irregular 'granular' bodies, the same sort of substance as found in the normal Sertoli cytoplasm. It is either continuous, except as interrupted by the nuclei, or broken by cavities, figures 3 B, 6, 8, 9, and 11.

In one respect it differs somewhat from the cytoplasm of the normal Sertoli tissue, or of the young tubule, in that it seems somewhat stringy or thready. This appearance is partly due to degenerate spermatozoan tails and partly to the tendency of the syncytial substance itself to form a more or less fibrous structure, which bears some resemblance to the fibers of connective tissue, but is much more delicate and less sharply defined. In figure 11, this characteristic is to be noted, particularly in the lower left-hand corner. In the normal tissue, when the spermatozoan heads have migrated well toward the lumen, this appearance is suggested to the observer, although the stringiness is much less well defined.

Other structures

From the quantitative point of view, the next structures to be described are the immature spermatozoa. These may be so abundant as to divide the space about equally with the Sertoli tissue or they may be relatively few. In the former condition they give to the tubule the appearance illustrated in figures 1 and 7. With higher magnification such a tubule appears to be filled with a more or less fibrillar mass intermingled with a granular substance, the whole taking any stain lightly, scattered through which the shrunken Sertoli nuclei are quite abundant (fig. 6). The fibrillar portions resolved into tails of spermatozoa, the heads of which, only partially formed, do not

stain prominently with nuclear stains. These immature spermatozoa are in bundles or groups, but so intermingled that it is out of the question to determine whether the number in each bundle is less or greater than normal.

In this type of degenerative condition, cavities appear which vary in size from one equal to two or three Sertoli nuclei to one whose diameter is half that of the tubule or even greater (figs. 6 and 7). Usually, however, with the increase in size of these cavities the immature spermatozoa disappear. There are also to be seen degenerate nuclei of a type other than those previously described. These are of various sizes, some smaller and some larger than the Sertoli nuclei. They stain yellowish in the iron haematoxylin and acid fuchsin preparations. Their contents consist of granular masses of unequal size, more or less densely aggregated. The medium-sized ones may have a clear space between such a unified mass and the nuclear membrane. No cytoplasm is discernible. Their identification is doubtful. They may be either remains of spermatid nuclei or degenerating Sertoli nuclei. That they may be the latter is indicated by the numerical relationships already referred to on page 98.

In this connection an individual difference in the case of one rat, no. 3554, may be noted. Degeneration seemed uniform but incomplete. In nearly every tubule the condition was that just described, that is, the tubules filled with the mixture of Sertoli tissue, the immature spermatozoa, and degenerate nuclei which stain yellowish. It is typically shown in figures 1 and 8. Scarcely a tubule was found showing degeneration to the degree illustrated in figure 5, and only a few with cavities as large as in figure 6. No spermatogonia, spermatocytes or spermatids were found. Along the basement membrane, however, in a very few tubules dividing cells are present, one or two to a tubule per section. These cells resemble dividing spermatogonia or the undifferentiated embryonic nuclei.

In the dividing cells just referred to the chromosomes and spindle are normal. One case of another type of dividing cell was seen, situated similarly, in which the chromosomes while abnormal resemble the first spermatocyte metaphase forms,

although the stage of division cannot be positively determined. No idiosome nor chromatoid body appears.

In the case of the other rats, cell division was rare in no. 3610, and abnormal; it was more abundant in no. 3896, and often normal. It was found in the cells along the basement membrane. Some cell division was observed in no. 3756, also along the marginal layer of cells. The chromosomes were normal and characteristic of spermatogonia in anaphase. In a tubule showing degeneration advanced to that typical of no. 3554, one such cell was found.

The degeneration in rat no. 3554 is much more uniform than in the others. The least uniform was no. 3756. Figure 3 shows that adjoining tubules may differ widely in this testis. Tubule A is practically normal; B has reached the stage a little later than that characteristic of no. 3554, while C is in the last stage observed. Only four tubules as nearly normal as A were found. This condition is therefore exceptional.

In no. 3896 more spermatocytes in early stages of development were found than in any other. These cells seemed to be normal. Growth has in some cases reached the leptotene stage. In the tubules showing these spermatocytes no immature spermatozoa were to be seen, but the wrinkled Sertoli nuclei were scattered freely in their characteristic cytoplasmic substance, and in some cases many cavities of large size appeared.

Occasionally pycnotic nuclei have been found in both the germinal and the interstitial tissue, but these are likely to be found in normal tissue. It is difficult to say whether the number of these in this degenerating tissue is more or less than the normal, as considerable variation exists in the normal. It has not impressed me as abnormal. In fact, I have been surprised that degeneration has taken that form so seldom.

The progress of degeneration

From the foregoing observations it would seem that degeneration had begun in the different individuals at different times or that different tubules reacted very differently to the diet. The condition found in no. 3554 would indicate that degeneration

had not begun until the spermatozoa had started to form, after which all the other germ cells had degenerated. Whether this lot of immature spermatozoa represent the first crop, which has persisted through the degeneration of the other germ cells, or the second or a later crop cannot be determined from the condition of the testes at death.

The uneven degeneration found in no. 3756 indicates that certain tubules were almost immune to the condition which brought about degeneration. These tubules are, however, very few indeed, so few that perhaps they may almost be neglected. At the same time the tubule shown in figure 3, A, shows all stages of spermatogenesis up to the spermatid formation. A similar stage in young material is normal.

In the case of rat no. 3896, in which complete degeneration of the germ cells and great reduction of the Sertoli nuclei were the rule, some tubules showed spermatocytes as far advanced as the leptotene stage. This last-named condition would indicate that for some reason these particular spermatocytes had been preserved during the degeneration of the Sertoli nuclei. It is not likely that they could have advanced much farther in their development. Certainly, the spermatozoa could not mature upon such pathological nurse cells.

The interstitial tissue

As previously noted, the interstitial tissue is relatively much greater than in the normal. This abundance is well shown in figures 1 and 2. The only cytological abnormality is that many of the glandular nuclei are slightly irregular in outline, often shrunken considerably. They react equally to the stain, while often in normal testes the reaction is quite unequal. Rat no. 3554 showed the shrunken and irregular nuclei much more abundantly than any of the others. In all the rats the amount of connective tissue seems about the same and quite normal. The endothelial nuclei are also normal. The interstitial tissue is unequally distributed, in some cases occurring in large masses. See figure 1 near the blood-vessel. Dividing cells are occasionally found.

OBSERVATIONS UPON THE MACDOWELL RATS

These rats, like the Osborne and Mendel lot, were in general about normal in development, with the exception of the testes of the five alcoholics, three of which, as shown by table 2, were under weight, one about normal, and one (no. 764) considerably over weight. In body weight based upon body length, there is a slight variation from the standard, from eleven under to fifteen grams over weight among the entire number. On the whole, the alcoholics ran a little over weight. The only two under weight were nos. 687 and 767, respectively eleven and five grams.

Degeneration in the MacDowell rats

The degeneration in these rats, while similar in kind to that of the Osborne and Mendel lot, varied greatly in degree in the different rats. Some testes were almost or quite normal, others slightly abnormal, while one showed almost as advanced a stage as that of the Osborne and Mendel rats. Unlike the first lot, however, degeneration of any degree had not extended equally throughout the organ. It was confined to certain portions only, like islands in normal tissue, these varying in size. They were most numerous and largest in no. 704. In fact, very little of this rat's germinal tissue was normal.

The final stage, when present, is confined to small portions of the tubule concerned, unless degeneration has progressed to a very advanced stage, as in no. 704, in which case considerable lengths of the tubule are involved. This last-named condition is shown in figure 7, the tubules in which are representative of the testis from which it was taken, no. 704.

In the early stage, the signs of degeneration are confined to small cavities which appear in the germinative wall, such as shown in figure 10, C. At a little later these cavities have enlarged and appear as in figure 7, C. At a later stage still, the cavities have enlarged, the germ cells are scattered through the lumen and are abnormal in various ways. The Sertoli nuclei usually remain near the basement membrane, but in advance stages are always to be found there. The Sertoli cytoplasm is shreddy and fills the

TABLE 2

Showing in A the body measurements, comparison of weight of testes with standard on body length, length of alcohol treatment, age when begun, age at death, and degree of degeneration of the MacDowell rats, and in B the corresponding data for the normal brothers

NUMBER	WEIGHT	LENGTH OF BODY + TAIL	LENGTH OF BODY	WEIGHT OF TESTES	STAND- ARD WEIGHT ON BODY LENGTH	AGE WHEN ALCOHOL WAS BEGUN	LENGTH OF TREAT- MENT	AGE AT DEATH	DEGREE OF DEGEN- ERATION
A									
	grams	mm.	mm.	grams	grams	days	days	days	
614	198	391	201	2.052	2.094	43	155	225	Slight
686	171	372	192	2.124	1.964	28	97	162	Slight
704	122	334	163	1.914	1.313	38	83	158	Extreme
725	142	347	176	1.963	1.609	28	83	148	Medium
764	191	370	196	1.778	2.951	28	77	142	Medium
B									
						Normal brother of			
618	279	422	220	2.075	2.567	614		225	Normal
687	269	410	222	2.574	2.609	686		162	Slight
707	211	380	202	2.822	2.181	704		158	Medium
727	185	350	192	2.240	1.964	727		148	Slight
767	210	394	206	2.049	2.267	764		142	Slight

interstices between the germ cells, just as in the Osborne and Mendel specimens, presenting the same appearance as that shown in figure 6, or, for the most advanced stages, as that shown in figure 5. The Sertoli nuclei are not as shrunken and wrinkled as in the Osborne and Mendel specimens, nor is the nucleolus so markedly different from the normal, seldom showing an appearance like that in figure 13.

In many places cells in various conditions appear, some normal, some polynuclear, some degenerating by pycnosis and others by a process in which the chromatin occurs in very small, lightly staining granules, yellowish in color with haematoxylin. Many of these may be identified as first spermatocytes, some in the growth stages and others in division. Giant cells occur frequently in the earlier stages of degeneration.

The tubules which show advanced degeneration are smaller in diameter than the normal, although this difference in size is not always as great as shown in figures 7 and 10.

The order of degeneration among the germ cells

In order of degeneration the spermatocytes seem to be the first affected, the spermatids next, and the spermatogonia last, although in some places the early growth stages of the first spermatocytes are found with spermatogonia, but no spermatids. In many places the spermatogonia persist along the basement membrane along with the Sertoli nuclei, but all other germ cells have disappeared. Under these conditions these spermatogonia nuclei are somewhat shrunken and wrinkled. I have not determined whether the second spermatocytes are more susceptible than the first.

The interstitial tissue

In these animals the quantity of interstitial tissue does not seem to be increased, nor have any shrunken nuclei been noted even in the most advanced degenerate conditions of the tubules. Further experiments are needed to demonstrate why this difference in this respect should appear between the two sets of rats when the germinal tissue is affected in a like manner.

CONCLUSIONS WITH REGARD TO THE TWO SETS OF RATS

The facts just set forth show that certain agents have a selective action upon the development of the germ cells. From the Osborne and Mendel rats we must conclude that for normal growth of these cells a diet containing a generous supply of the water-soluble vitamine is an essential. Just how much remains to be determined by further experiment.

From the MacDowell rats studied, a conclusion is not so easily drawn since two factors entered into their history. They were all subjected to a reduced diet for fifteen days, beginning when they were ten weeks of age, while half of them were also subjected to the fumes of alcohol for a period long enough to

produce complete intoxication daily during a time varying from seventy-seven to one hundred and fifty-five days. Neither of these treatments interfered with these rats' producing offspring, although the whole group of alcoholics from which they were taken showed a considerable reduction in fertility (MacDowell and Vicari, '17), to the extent that twenty-nine pairs of normals produced 300 young, while during the same time thirty pairs of alcoholics produced only 108 young. How far the male is responsible does not yet appear, but it is clear that in the ten rats of the series which came to me the degeneration is consistently greater in the alcoholics than in their control brothers, both from the extremes and from the average, as is brought out in table 2.

Under the circumstances, it is unwise to draw any general conclusions as to the cause of the degeneration in these MacDowell rats. What does seem clear from a comparison of the two lots under consideration is that similar conditions of degeneration may arise in the testes of rats subjected to widely different treatments, and that the immediate causes affecting growth and cell division in the germ cells may be identical.

DISCUSSION

The discussion is divided naturally into anatomical and physiological considerations. With respect to the former, the chief interest centers about the type of degeneration and the light it throws upon the Sertoli tissue.

The type of degeneration is not new. Regaud ('01) describes similar conditions in the tubules of the white rat near their distal extremities, but does not state a cause. The same author ('10) and Barratt and Arnold ('11) find a similar type of degeneration in mammalian testes which have been subjected to x-rays. Colwell and Russ ('15) have assembled the effects of x-ray treatment upon tissues and note nothing different in connection with the testis. They quote chiefly Regaud and the work of Barratt and Arnold, just referred to, and reproduce the latter's figure 30 as their figure 40.

Regaud ('10) worked with rabbit, guinea-pig, mouse, cat, and rat, and obtained essentially similar results with all. His figure (from cat) shows a condition practically identical with my figure 5. Barratt and Arnold ('11) used the rat. Their figures 30 and 31 represent the same phenomena as my figures 11 and 6, respectively. The descriptions given by the three authors just quoted indicate that there is no difference in the final histological picture—total destruction of the germ cells, the Sertoli tissue alone remaining within the tubules. Barratt and Arnold claim an increase in the number of Sertoli nuclei found in the final stages as compared with the earlier, but give no data upon which such a conclusion has been based. My own observations indicate the reverse, as already noted under 'Observations.' Barratt and Arnold indicate that this increase is accomplished by amitotic cell division, a process which I believe does not exist in the tissues which I have examined, either Sertoli or germinal.

Barratt and Arnold note a diminution in size and edematous state of the testes, the clear fluid running freely upon incision of the tunic, two conditions which I found in the Osborne and Mendel rats.

With regard to the order of cell degeneration, Regaud ('10) finds that the very young spermatocytes and the last generation of spermatogonia are very sensitive so that they are entirely destroyed. Further than this he does not analyze the order. He does note in the final stage the persistence of certain large cells long the basement membrane, which resemble the "mâles ovules, or better the oviform spermatogonia of the prepubertal animal." These are probably the same cells to which I have referred, and which I am inclined to interpret in the same way.

Barratt and Arnold ('11) indicate that the second spermatocytes are the first cells to begin degeneration, as they state that amitotic division was observed in them twenty-four hours after the application of x-rays (p. 261), while in the first spermatocytes necrosis was not observed until after the third or fourth day, necrosis of the spermatids begins after the fourth day and is marked by the ninth day, whereas the spermatogonia ceased

to be recognizable after the fourth day. These observations would indicate that the spermatids were the most resistant. These changes are not in the same order as I found them in the MacDowell rats, where the spermatogonia are the last to degenerate.

The Sertoli tissue as a syncytium

With regard to the Sertoli tissue, my studies confirm Regaud's conclusion ('01) that this tissue is a syncytium. In order to satisfy myself that this is its normal state I reexamined many slides of well-fixed rats testis which I had used in my work on spermatogenesis, and studied for the first time the Sertoli nuclei and cytoplasm with great care. I found many places where the cytoplasm could be traced from one Sertoli nucleus to an adjoining one without encountering a cytoplasmic wall. In some poorly fixed material prepared while I was experimenting upon technique, I did find suggestions of a wall, but such appearances seem better interpreted as a thickening of the cytoplasm incident to the shrinking action of the fixative. This condition is quite likely to occur when Flemming's fluid is used, if during dehydrating or infiltrating too abrupt changes are made in the strengths of the reagents.

My series of developing tubules enabled me to trace the Sertoli tissue from the very young tubules in which there is only one type of nucleus present up to the condition where all the various stages of the germ cells are present. In the young tubule a syncytium is plainly the rule. One can readily see when the first cytosomes are differentiated. These are early growth stages of the first spermatocytes, and are shown in figure 5 of my spermatogenesis paper (Allen, '18). As these cells continue to increase in number, they differentiate cytoplasmic walls, but I have not been able to find any Sertoli cytosome thus developed.

The distinctive nucleolus of the Sertoli cell enables one to be certain whether, in a particular case, he is dealing with a germ or nurse cell. Consequently, determination of the stage when the Sertoli cell first differentiates is an easy matter. It does

not appear until after the first spermatocytes are well developed. In fact, I did not find it until the spermatids had formed. When I made this observation I was unaware of Regaud's statement in his 1901 paper: 'La première apparition des noyaux de Sertoli dans l'épithélium séminal a lieu, chez le Rat, au moment de la puberté, ou plus exactement au moment où sont formés les premières spermatozoïdes normaux' (p. 374, 11, 15 to 19).

By the time this stage of growth is reached the germinative wall is closely packed with germ cells, as shown in figure 4, so that the determination of cytoplasmic walls in Sertoli cells is very difficult.

It would appear that the original syncytium of the young tubule persists as such, within which the germ cells lie enmeshed. In the degenerate conditions described in this paper and by Regaud, and Barratt and Arnold, and others, no new syncytial tissue is developed, but the Sertoli syncytium is simply revealed by the loss of the germ cells, which under normal conditions obscure it.

The Sertoli nucleolus

The character of the Sertoli nucleolus needs further consideration. Previous reference has been made to the duplex nature of this body. Under normal conditions it consists of a large nearly spherical body close beside which is a much smaller one, also approximately spherical. For the sake of convenience, I shall refer to this latter as the paranucleolus.¹ It is not wholly unique with the Sertoli nucleolus, as a similar body is to be seen in well-fixed preparations of first spermatocytes in their late growth stages. In my paper on spermatogenesis (Allen, '18), the nucleolus is shown as a simple spherical body in figure 27. The cell from which this drawing was made had been fixed in Bouin's fluid, not by the fluid which gave the best fixation, B-15. Before publishing the paper to which reference has just been made I did not study the nucleolus carefully in my preparations fixed with the improved fixative. Since then, in connection with these Sertoli studies, I have discovered from this better-fixed material that its true structure is bipartite, and

unequally so. However, in the first spermatocytes the smaller body is more closely united with the larger than in the case of the Sertoli nucleolus. A similar body in connection with the nucleolus has been described by Miss Carothers ('13) in the germ cells of certain orthoptera. Further comparative study is needed to establish a common identity.

Regaud ('01) figures on plate 7 several Sertoli nucleoli in which the paranucleolus, as I have described it, appears distinctly. See his figures 19, 23, 38, and 39. He states that it stains blue with haematin and safranin after Tellyesniczky fixation, while the larger portion stains red. He states also that in Sertoli nucleoli fixed in bichromate acetic the nucleolus is always 'purely safranophile,' or if haematin is used it appears 'pale violet gray.' 'Il est toujours homogène et parfaitement sphérique' (p. 302, 11, 31 to 34). It is thus clear that he does not regard the paranucleolus as a part of the nucleolus. He figures also one or two small bodies staining similarly to the paranucleolus which lie near the nuclear membrane, within the nucleus, such as I show in figures 15 to 17. I am inclined to think that he regards the paranucleolus as one of these which sometimes happens to lie beside the nucleolus proper. He states also, page 303, 11, 87 and 18, that the presence of two equal spherules in close contact with the nucleolus at the two extremities of one of its diameters is quite exceptional in the white rat. I have found such an arrangement of these bodies very common in the spermatogonia of the albino rat. In fact, so common that I am inclined to the opinion that perhaps it is the rule in a certain stage of their development, but more study is needed before positive statement may be made.

I have not employed the bichromate acetic fixative except in one case, and it gave such poor results compared with B-15 that I did not follow it up. Therefore I cannot speak of the differential staining reaction of the larger and smaller portions of the nucleolus from my own experience. With safranin after Flemming or B-15 both nucleolus and paranucleolus stain alike, as they do with iron alum haematoxylin. I have observed, however, in some Sertoli cells fixed with B-15 and stained with

iron alum haematoxylin that the paranucleolus does not always stain with the same density. The associated activities of the Sertoli cells have not yet been studied fully, but there seems to be some evidence to indicate a connection between these and this unequal staining reaction. At any rate, the evidence is sufficient to warrant further study under different fixatives and stains, and with varying physiological conditions of the rat.

I have previously called attention to the abnormal Sertoli nucleolus in the last stages of degeneration in the Osborne and Mendel rats, shown in my figures 11 and 13. This condition does not seem to have been noted by other workers, so far as I have read. It is shown, however, in one Sertoli cell in Regaud's figure of the x-rayed cat tubule ('10). It is not figured in any of Barratt and Arnold's Sertoli nuclei, although in other respects they duplicate those in my material.

The interstitial tissue

Barratt and Arnold ('11) do not report upon interstitial tissue from their own experiments, but call attention to the work of Bergonie et Tribondeau in 1904 upon the white rat, stating that they report hypertrophy of this tissue a month after the last exposure to x-rays, a condition which persisted two months later.

Physiological considerations

I shall not do more than call attention to some matters of interest in this connection. A full discussion would be premature, under all the circumstances. There would seem to be a similar physiological condition induced by such widely different agents as x-rays and the lack of a plentiful supply of water-soluble vitamine in the diet. This similarity is manifested in both the degeneration of the sex cells and the hypertrophy of the interstitial tissue. In the case of the Osborne and Mendel rats the hypophysis was smaller than normal (from measurements made by Donaldson on five rats from this same series). Regaud ('01) reports degeneration in the distal portions of the tubules, but gives no cause for it. The MacDowell rats, both alcoholics

and four out of the non-alcoholics, show degeneration to greater or less extent; all of the alcoholics more than the others. All ten of these rats had been starved briefly (from seventy to eighty-five days of age), but all had lived for more than two months after that. Aside from this apparently slight detrimental influence, we know of no cause for the degeneration in the non-alcoholics. If one were present, it would appear that the alcohol aggravated its deleterious effect upon the germ cells.

That this degeneration is not an old-age phenomenon is proved conclusively by the conditions found in many rats of the Wistar colony older than any of the MacDowell lot, and one of about six years of age. In none of these is this type of degeneration to be seen. I have prepared a series of testes of rats from the colony at various ages—standards, hybrids, and inbreds—and none of them indicate that there might be an individual variation along this line.

It would appear, therefore, that experiments upon comparative diets would throw light upon the physiological problems raised by the conditions discussed in this paper. Examination of all the organs, especially those of the endocrine system, would be desirable in the case of such animals.

SUMMARY

1. Reduction in the quantity of water-soluble vitamine in the diet of rats results in total degeneration of all the germ cells, but does not interfere with growth and development in other respects. The Sertoli tissue persists.

2. In the male this atrophy of germ cells is accompanied by hypertrophy of the interstitial tissue.

3. The type of degeneration in the male germ cells is similar to that produced by x-ray treatment of the testes directly.

4. A similar degeneration of male germ cells has been observed in a group of rats, part of which were subjected to prolonged alcoholization. The degeneration was found to a less extent in all but one of their five brothers not alcoholized. In this group hypertrophy of interstitial tissue was not observed.

5. Examination of this degenerated tissue and more careful study of normal, well-fixed tissue confirms Regaud's conclusion that the Sertoli tissue is a syncytium.

6. The nucleolus of the Sertoli cell under these degenerated conditions appears to be an equally bipartite, instead of as normally an unequally bipartite body.

7. The interstitial tissue is much increased in quantity in the rats fed upon a reduced water-soluble vitamine.

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PLATES

The photomicrographs were made by the author with combinations of lenses suitable for the magnification desired, figures 11 to 14 with oil immersion. Figures 1 to 6 are reproduced at the original size. Figures 7 to 14 are reduced one-third from photographs which were made by enlarging the original negatives three times by projection. The drawings were made by tracing the outlines on the backs of photographs, transferring these to the drawing-paper by a sheet of carbon, and filling in the details with lithographic pencil.

The sections were cut at 7μ or 10μ and stained with iron haematoxylin and acid fuchsin. All are from the Mendel and Osborne rats except figures 7 and 10, which are from the MacDowell series, and figures 14 to 17, which are from an adult normal rat from the Wistar colony.

PLATE 1

EXPLANATION OF FIGURES

1 and 2 From nos. 3554 and 3610, showing the difference in appearance between the two. Limited portions of these are enlarged in figures 8 and 9. The exceptionally large quantity of interstitial tissue is well shown in figure 1. This rat was injected with the fixing fluid, a process which preserves more nearly the normal relationship between tubule and interstitial tissue and holds the blood-vessels distended. $\times 30$.

3 From no. 3756. Three adjoining tubules showing unequal stages of degeneration, A, B, and C. $\times 150$.

4, 5 and 6 are enlarged views of the tubules shown in figure 3. $\times 300$.

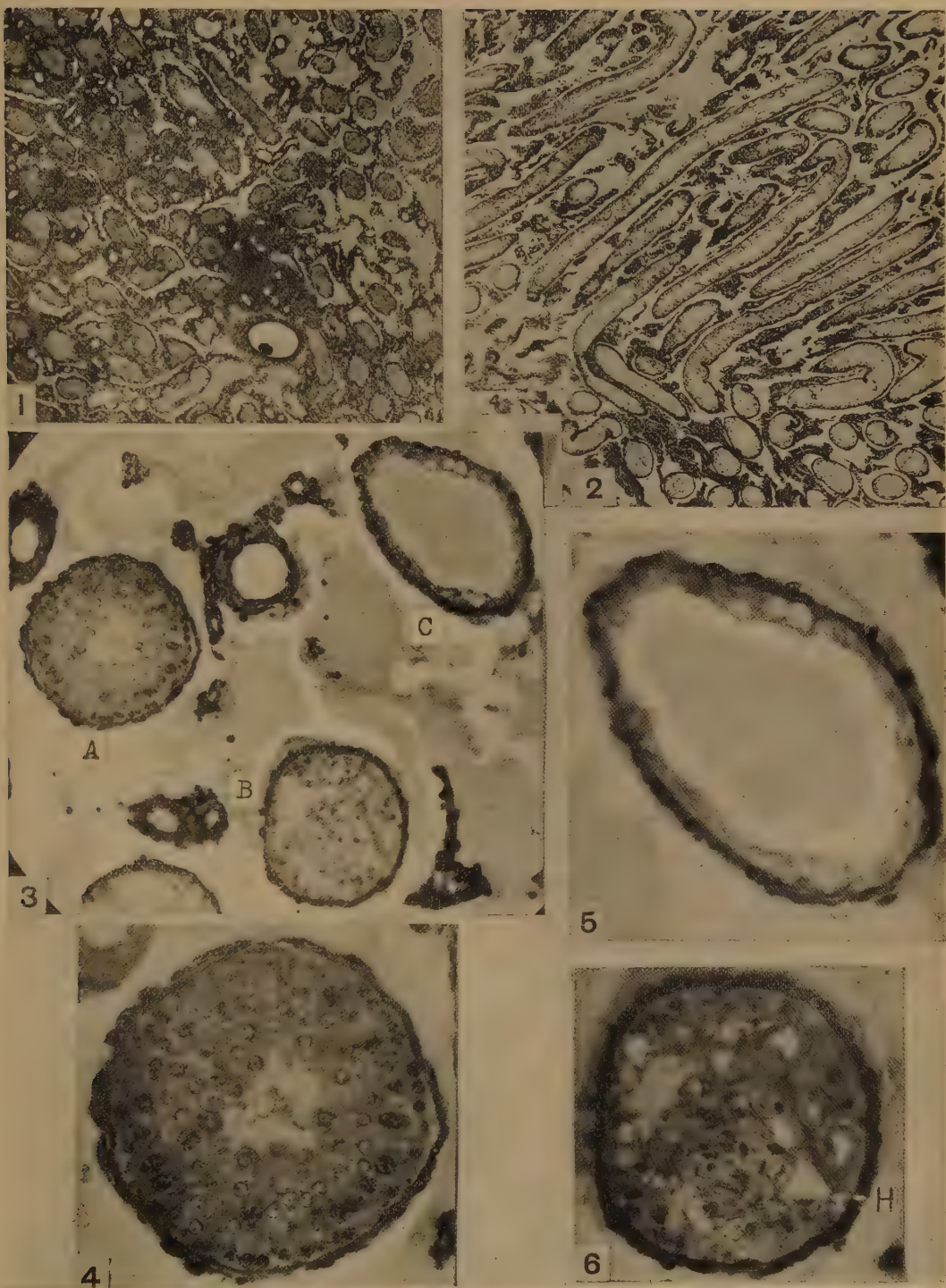


PLATE 2

EXPLANATION OF FIGURES

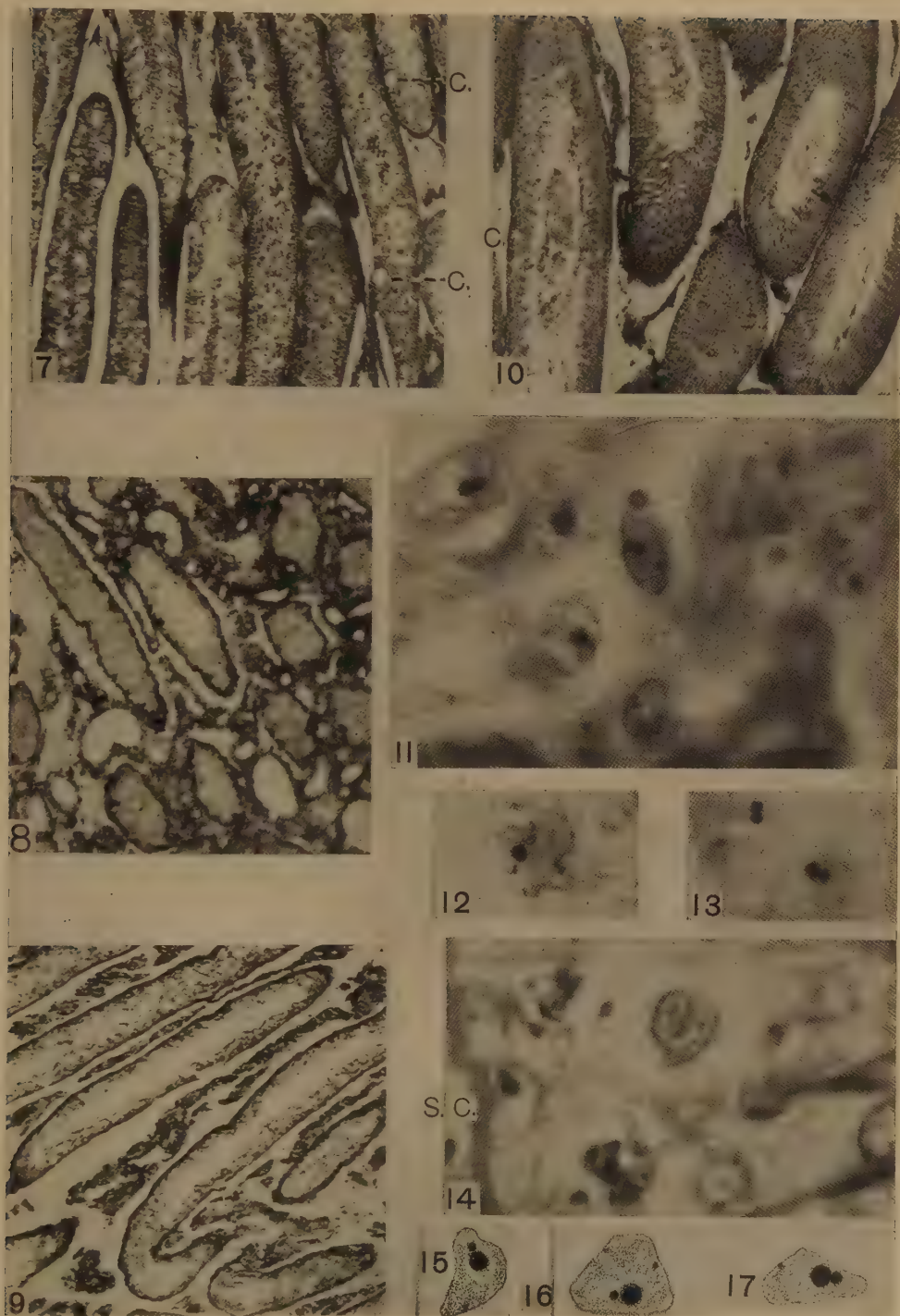
7 and 10 From MacDowell rats nos. 704 and 764, showing the difference in degree of degeneration; both alcoholics. *C*, cavities. These are very distinct in figure 7, while almost unnoticeable in figure 10. The germinal epithelium is practically normal in figure 10, while in figure 7 it is nearly as abnormal as in figures 8 and 9. $\times 60$.

8 and 10 Enlarged views of portions of figures 1 and 2, showing details of the germinal epithelium a little better. $\times 60$.

11, 12 and 13 These figures show the wrinkled nuclei and the characteristic diploid nucleoli of the Sertoli tissue. Figure 11 fairly represents the Sertoli syncytial cytoplasm. A grooved nucleus is shown in the extreme right; two others near the lower center. $\times 1500$.

14 Small portion of normal epithelial wall of tubule, showing at *S. C.* the Sertoli nucleus. At the right of this nucleus the Sertoli cytoplasm extends to the spermatozoan heads at the extreme right, passing between first two spermatocyte cells in early prophase and two spermatids, respectively, as one traces it from the nucleolus to the spermatozoan heads. $\times 1500$.

15, 16 and 17 Three nuclei of Sertoli cells, showing the paranucleolus in each and the small bodies which stain similarly but lie near the nuclear membrane. Figure 15 is the same nucleus as is shown in figure 14. $\times 1500$.



RALPH EDWARD SHELDON¹

1883-1918

IN MEMORIAM

ROBERT RETZER

By the death of Ralph Edward Sheldon the University of Pittsburgh lost one of its most constructive and conscientious professors, the American Association of Anatomists a member who ranked first among the present generation, and his colleagues a loyal and true companion. In the University he was a counsellor whose opinions were valued by his seniors, and as a teacher he brought out the best qualities in his students. His investigations in the field of neurology gained him a national reputation. We mourn his departure the more because he left us so suddenly with his promising life's work but half completed.

Doctor Sheldon was born at Lisle, New York, on March 28, 1883, the son of Herbert Clayton Sheldon and Rosalia Reed Sheldon, who both show a long line of New England ancestry that can be traced on one side to John Alden. As a boy he was exceedingly fond of outdoor sports, and kept up this interest until he went to Harvard. He then became so absorbed in work that he gave it up, but promised himself a renewal of these activities when he had completed the book.

After attending the public schools, he entered Cornell University in the College of Forestry, but as this College was discontinued after the first year, he took the regular Arts course. He continued his interests in this field, as is testified by the fact that during the summers of 1902 and 1903 he was assistant in the United States Forest Service. Voluminous notes taken at these expeditions together with numerous books and reprints also bear testimony to this interest. This probably would have become an avocation had he found any time to spare. Three

¹ An address, in memory of Ralph Edward Sheldon, presented at the thirty-fifth session of the American Association of Anatomists, convened at the University of Pittsburgh, April 17, 18, and 19, 1919.

years after matriculation in 1904 he received the A.B. degree and a scholarship in Neurology which led to a Master's degree in 1905. He then was rewarded the Goldwin Smith Fellowship in Neurology, which he held during the year 1905-06. He was known as a hard worker, and throughout his college career he earned money to pay for his education. He took most careful notes in all his studies, which were of the most varied kind, including Latin, Greek, French, German, surveying, botany, chemistry, and physics. In later life he acquired a reading knowledge of Spanish, Italian, and a little Dutch.

In the summers of 1904 and 1905 he was Assistant in Zoology at Cornell. While at Cornell he worked for Professor Wilder and became interested in Neurology. It is unquestionable that this association exercised the greatest influence in his career. Having built a small summer home in Ithaca, he went back to work at Cornell University many summers. From 1906 to 1907 he worked under an Edward Austen Fellowship under Professors Mark, Parker, and Castle at Harvard and received the S.M. degree. He then received a fellowship in the University of Chicago, but a vacancy occurring he was appointed Assistant in Anatomy. After one year he received a Ph.D. degree under the direction of Professor Herrick, with the thesis, "The Olfactory Tracts and Nerve Centers in Teleosts." He was appointed Associate in Anatomy in 1909, but feeling the necessity of a salary that was adequate to meet the needs of a growing family he accepted an offer of Professor Cohoe to become Assistant Professor of Anatomy at the University of Pittsburgh. After three years he was appointed Associate Professor and in 1914 Professor and Head of the Department.

At times he was discouraged because he soon realized that it was almost impossible to combine research work with the many duties that fell upon his shoulders, but he nevertheless not only kept up his scientific interest, but managed to write a creditable number of scientific papers. Had he not undertaken to write a textbook on Neurology the anatomical world would have been enriched considerably by many more contributions.

He was a Fellow of the American Association for the Advancement of Science and a member of the American Medical

Association, American Association of Anatomists, American Physiological Society, American Society of Zoologists, American Society of Naturalists, Anatomische Gesellschaft, Society of Biological Research of the University of Pittsburgh, Allegheny County Medical Society, Pittsburgh Neurological Society, and of the Gamma Alpha, Phi Rho Sigma, and Sigma Xi Fraternities. He was very active in the Cornell Alumni Association and for years was secretary of the scholarship committee of Western Pennsylvania. He was also a member of the Harvard and of the Chicago Alumni Associations.

At the last meeting of this Association, Doctor Sheldon extended the invitation of this department for the next meeting. He was keen to show what this department had accomplished in the few years since he had taken hold of it. It should be remembered that when he came here less than ten years ago this building had not been built. The school was in the process of reorganization and presented all the crudeness which was so common in all of our proprietary medical schools at that time.

The physical equipment of the Department of Anatomy of the University of Pittsburgh Medical School as you see it to-day is essentially the work of Doctor Sheldon. When one reviews his brief career before Doctor Sheldon came to this institution, one cannot but marvel at his remarkable ability to combine what he found best in other institutions, organize, equip, and administer a department in a manner that would have been a credit to a man of twice the amount of Doctor Sheldon's experience. While this accomplishment might have been attained by many a man whom we are wont to speak of as having an executive ability, it is most remarkable that through it all Doctor Sheldon never allowed himself to be so overwhelmed by the multifarious duties that he could not continue the prosecution of research. It is true that we find in looking over his publications that there are a number of papers that deal strictly with matters of laboratory equipment, yet I have found among his documents innumerable notes on scientific observations he had made that needed but little more work in order to put them before the public. Here we must not forget that he was a young man and had standards

of perfection which were difficult to attain. With years we gradually learn that careful observations, although limited in scope, are in themselves valuable to others besides ourselves and merit publication. As I say when we scrutinize his list of publications, we may doubt whether he may not have fallen by the wayside as is the fate of so many men of promise who undertake administrative duties. One needs but look carefully at some of his chapters in his text-book, however, to come to a very different conclusion. Observation of facts and phenomena is but the framework—the body—of Science. It is the elucidation and the correlation of these observations that give Science life. Sheldon not only observed keenly, but he analyzed and criticised keenly. The major part of his book is not a paraphrase of the writings of other neurologists, but a critical analysis of the most recent work on the subject.

He took a remarkably keen interest in the students' welfare and progress. Although in the last years he gave but one course, that of neurology, he always inquired of his staff, how each student was progressing, and at least once a year he interviewed each student personally, giving a word of cheer or encouragement whenever he could, but showed no leniency in meting out punishment to offenders. In this connection I challenge anyone who will say that there is any medical school where the scholarship and the personal fitness of its students are more carefully scrutinized and where students who do not maintain a given standard are either forced to withdraw from the school or repeat the entire year. We owe this primarily to Doctor Sheldon. It was he who set the standards of attainment for the first-year students; it was he who brought the names of the delinquents before the executive authorities, and it was he who always unflinchingly stood for the best. He may have appeared to some as a heartless judge, but yet beneath that sternness was a melting heart. He expected the student to do well the work allotted to each course. If he failed to do so, it meant repetition of the course, and this frequently entailed the repetition of the whole year. This seemed to him such a hardship that he voluntarily without any financial reward gave summer courses for those

students who were unable to attend regular summer schools in order to enable them to continue with their classes.

When war broke out Doctor Sheldon, like all true patriots, wanted to tender his services. He was urged by Major Bagley to apply for a commission. He was torn between two duties, and chose the harder one—he stayed at home. He, nevertheless, offered the services of this department to the Council of National Defense and began some very important work on staining methods for formalin-fixed material. The first report was sent to Washington a few weeks before his death. The methods he devised were then used in several laboratories in the neuropathological service.

We meet here in the department which at every turn bespeaks of his activities. In the dissecting-room the tables, book racks, museum cases, cadaver tanks, all were designed in every detail by him. In the histological laboratory the desks with the interchangeable units of drawers, slide holders, and reagent racks, in the private rooms the slide and stock cases were built according to his specifications. A case full of charts, nearly all of which were drawn under his direction, give further evidence of his zealous attempts to serve the student and the medical school. The anatomical library was built up by him, and considering the short time he was at the helm, the war conditions, and the not too ample funds, it shows better than anything else how he had built for permanence. The library is not filled with antiquated text-books, but with most of the current anatomical periodicals. What cannot be seen but is nevertheless felt by those who have been associated with him is the highly scientific and moral atmosphere which he had created in the nine years he was a teacher in this institution.

He was a most indefatigable worker and a most meticulously careful man, and as is so often the case with men of this type, he expected everyone else to do as much as he did. His employees dared not shirk their tasks. Every day he quickly passed through all the rooms of the department and noted everything that was done or undone. It should not be judged from this that he attempted to run the department single-

handed. It was his aim to train the members of his staff to do the right kind of work and then give them responsibilities. He conferred and directed but allowed his assistants to work out the details. The result was that these men acquired an independence of thought and action as is rarely found in an institution that is guided by a youthful hand.

His activities did not cease with the work he found to do in his department. His unbounded enthusiasm and determination caused his colleagues to throw many more burdens on his shoulders. One has but to look over the documentary file of this department to realize that not one single phase of medical school and university administration had escaped his personal attention. Elaborate tabulations of curricula, schedules, budgets, staffs, and equipment of every prominent medical school in the country were orderly filed. Though the youngest professor, he was chosen the representative at large of the medical school to sit in the council of the University. Committee meetings upon committee meetings consumed his energy, of which he seemed to have an absolutely unlimited amount. The reason he devoted so much time to these activities is well expressed in a letter written on December 1, 1915.

It seems to me that every individual in the service of the University should be made to feel that the interests of the University must always be paramount to those of the individual. A department for instance is not the personal possession of its chief. He is given certain rights, privileges and facilities in order that he may use these for the interests of his school and the University as a whole. To the extent which he fails to do this, he is not living up to his obligations to the institution. I feel, of course, that a considerable leeway must be given in the interpretation of this in order that full advantage may be taken of individual initiative, and that, therefore, it is not possible to lay down hard and fast rules regarding the conduct of individual departments. If it could, however, be indicated to every member of the teaching staff that loyalty to the department, the school and University would be an important factor in evaluating his work, and if the individual could feel that all were on an equal footing in this regard, I believe it would do more than anything else to establish, in the institution, a spirit of affection and loyalty. In this evaluation a wide acquaintance of the work of all departments of the University and high ideals of attainment are necessary in order that work of individuals and departments

along widely divergent lines shall be fully appreciated and that substantial work for the benefit of the institution receive due credit as compared with that which happens to receive publicity.

As previously stated, he was intensely interested in scientific work, and his published papers show that he was as capable a scientist as he was an administrator. In his paper on "The Nervus Terminalis in the Carp," he gave the first account of this nerve in the teleosts. "The Olfactory Tracts and Centers in Teleosts" is his magnum opus of scientific research. It represents the most detailed and accurate analysis by an anatomical method of the functional localization of tracts and centers of any vertebrate hitherto described. He carried the work to the last refinement permitted by a combination of all available strictly anatomical methods, and the work is a model of its kind.

It was only after his death that I learned that Doctor Sheldon was very much interested in Chemistry. It is therefore not surprising that the interest in this science should have led him to apply it to neurology. In "The Reactions of the Dogfish to Chemical Stimuli" he showed that the skin of fishes is exceedingly sensitive to some chemicals, even more so in some cases than the taste-buds. In "The Sense of Smell in Selachians" and "The Sense of Smell in Teleosts" he intended to test physiologically the anatomical work of his doctor's thesis. These five papers represent an ideal of combined anatomical and physiological work on a definite program such as has rarely been attained by any investigator.

"The Phylogeny of the Facial Nerve and Chorda Tympani" is a valuable summary illustrating the value of comparative study in solving vexed problems of mammalian anatomy. Phylogenetic history, experimental physiology, and pathological anatomy are brought to bear on the problem of human peripheral conduction paths bringing about noteworthy results in clarifying practical problems of surgery. He summarized in "The Paraffine-Weigert Methods for the Staining of Nervous Tissue, with Some New Modifications" his own and many others' extensive experience in the difficult problem of getting the most possible out of the Weigert method in the study of both human

and comparative brains. It is one of the most helpful contributions to technique in the literature.

When he was but twenty-six years old he projected a text-book on Neurology. This undertaking was his life of the last two years and it was his death. The book was practically written with 950 pages of manuscript six years ago. Being inexperienced, he thought it a comparatively easy matter to have the illustrations made and the manuscript set to type. Before long he was sadly disillusioned.

When I came to this department a little over two years ago I was asked to look over a few chapters of the book. I was amazed to find that the book which I had expected to use for my classes the previous year had not progressed any farther, but it did not take me long to find the cause of this delay. It was not teaching, it was not administrative work that was the cause of the delay, but his endeavors to do the impossible—write a perfect text-book. His publishers rightly urged and urged. Letters, telegrams, and representatives called for haste. He finally realized that perfection was not attainable and more rapid progress was made. He was relieved of most of his teaching duties, and then it was book from early morning to late at night. His almost indecipherable handwriting made it difficult for his stenographers, and to facilitate matters he used the dictating machine.

This is not the place to review the book. It represents the first attempt to present in a text-book the subject of Neurology from a functional point of view. It is intended to give the medical student a broad conception of the fundamental principles that underlie the structure and function of the nervous system, but at the same time pointing out the paths for future investigations. The reason he undertook this work may be best stated in his own words found in the preface written many years ago.

To the older anatomists the nervous system was only an anatomical structure, to be dissected out and studied in relation to the other organ systems, such as the bones, blood-vessels, muscles, etc. This attitude of mind led to the development of a school of neurologists, both human and comparative, who devoted themselves to the study of the gross

morphology of the brain and peripheral nervous system in the most minute detail, identifying and comparing every depression, protuberance, and membrane and their relations to the surrounding tissues of a different kind. Although his interests are different, the worker of to-day, with a wealth of technical methods at his disposal, must always look with amazement and admiration at the results which these men secured with the crudest of methods.

At present there is no treatise available, which, in addition to the gross relations, will give to the student of anatomy, neurology, medicine or to practicing physicians, a complete presentation of the functional relations of the nervous system. This book represents an endeavor to fill this gap and to present in an adequate fashion for such workers the gross and microscopic anatomy of the nervous system with all the more important functional relations.

I cannot help but speak from the very depth of my emotions. The two years that I was associated with him I treasure as two of my most valuable ones. There was no barrier between us and I felt I had his utmost confidence as he had mine. We had similar ideals and it was a distinct pleasure to be able to work harmoniously by his side. Not once during these years did we part in an argument or discussion but that I felt a greater admiration for his personality. The sternness which he felt he had to assume on account of his youth would melt in a smile that made you feel happy in his presence. He seemed to fairly radiate good-will and energy.

I am glad that a month before his death I had coerced him to take an auto trip with me through Maryland. We both were boys again. Oblivious of everything that might burden the heart of a man we were for two days as care-free as the birds that flitted about. He vowed that he never had had such a good time and promised that before the summer was over he would take his family over the same trip. A month later and his bright career came to an end. On Sunday he worked to get off some drawings to the publishers. On Monday he felt 'grippy' and thought he would rest up a bit. On Tuesday morning, July 9, he got up and thinking that it might dispel his lassitude he took a hot bath. When he stepped out of the tub, weakness was manifest in one foot. Before the hour was over both feet were paralyzed. The paralysis rapidly crept upward, and by midnight his spirit had departed. It is curious irony of fate that Dr. Sheldon should have succumbed to a nervous dis-

ease, Landry's acute ascending paralysis, the nature of which we understand so little. It is probable that he thought the paralysis a transient one. He was hopeful to the end.

The influence Dr. Sheldon exerted during his brief career will live a long time. His scientific work has placed him among those that have given substantial contributions to the advancement of knowledge. The influence he exerted as an educator will long be felt by his students and by his students' students. He founded a department which it is hoped will ever reflect credit to his name. It is also hoped that, realizing the debt the University and the anatomical world owes him, a way will be found to bring before the world the book which will always stand a monument to industry.

PUBLICATIONS

- The participation of medullated fibers in the innervation of the olfactory mucous membrane of fishes. *Science*, vol. 27 pp. 915-916. 1908.
- An analysis of the olfactory paths and centers in fishes. *Proc. Assn. Amer. Anat.*, *Anat. Rec.*, vol. 2, no. 3, pp. 108-109. 1908.
- The nervus terminalis in teleosts. *Proc. Assn. Amer. Anat.*, *Anat. Rec.*, vol. 3, no. 4, pp. 257-259. 1909.
- The nervus terminalis in the carp. *Jour. Comp. Neur. and Psychol.*, vol. 19, no. 2, pp. 191-201, figs. 1-7. 1909.
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- The phylogeny of the facial nerve and chorda tympani. *Anat. Rec.*, vol. 3, no. 12, pp. 593-617, figs. 1-6. 1909.
- The sense of smell in selachians. *Jour. Exp. Zool.*, vol. 10, no. 1, pp. 51-62. 1911.
- Some new laboratory furnishings. *Anat. Rec.*, vol. 5, no. 10, pp. 483-490, pls. 1-4. 1911.
- The olfactory tracts and centers in teleosts. *Jour. Comp. Neur.*, vol. 22, no. 3, June, pp. 177-255, pls. 1-42. 1912.
- The sense of smell in fishes. With G. H. Barker. *Bulletin of the U. S. Bureau of Fisheries*. Vol. 32, 1912, Document No. 775, May 3, 1913, pp. 35-46.
- Some new dissecting-room furnishings. *Anat. Rec.*, vol. 7, no. 10, pp. 369-370. 1913.
- Paraffine-Weigert methods for the staining of nervous tissue, with some new modifications. *Folia Neuro-Biologica*, Bd. 8, Nr. 1, S. 1-28. 1914.
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PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

THIRTY-FIFTH SESSION

*Medical School of the University of Pittsburgh; Pittsburgh,
Pennsylvania*

April 17, 18 and 19, 1919

THURSDAY, APRIL 17, 9.30 A.M.

The thirty-fifth session of the American Association of Anatomists was called to order by President Robert R. Bensley, who appointed the following committees:

Committee on Nominations for 1919: Professor J. Playfair McMurrich, Chairman; and Professors George S. Huntington and Florence R. Sabin.

Auditing Committee: Professor Eliot R. Clark, Chairman; and Professor Frederic T. Lewis.

The morning session was devoted to the presentation of scientific papers followed by a paper in memory of the late Professor R. E. Sheldon, presented by Professor Robert Retzer. Following this, the final feature of the morning programme was an address by the President of the Association, Professor Bensley on "Anatomy, a Science or a Curriculum?"

FRIDAY, 11.30 A.M. ASSOCIATION BUSINESS MEETING, PRESIDENT ROBERT R. BENSLEY, PRESIDING.

The Secretary reported that the minutes of the Thirty-Fifth Session were printed in full in *The Anatomical Record*, volume 14, number 1, pages 19 to 23, and read the minutes as printed. On motion, seconded and carried, the minutes of the Thirty-fourth Session were approved by the Association as printed in *The Anatomical Record*.

Professor E. R. Clark reported for the Auditing Committee

as follows: The undersigned Auditing Committee has examined the accounts of Doctor Charles R. Stockard, Secretary-Treasurer of the Association of Anatomists and finds the same to be correct with proper vouchers for expenditures and bank balance on January 8th, 1919, of \$211.59.

(Signed) ELIOT R. CLARK,
FREDERIC T. LEWIS.

The Treasurer made the following report for the year 1918:

Balance on hand December 19, 1917, when accounts were last audited.....	\$303.83
Receipts from dues 1918.....	2391.86
Total deposits.....	\$2695.69
Expenditure for 1918:	
Expenses Secretary-Treasurer. Minneapolis Meeting.....	\$132.91
Postage and Telegrams.....	48.12
Printing and Stationery.....	45.24
Collection and exchange on drafts.....	1.73
Stenography, typewriting.....	45.60
One check returned and debited.....	7.00
Wistar Institute, subscriptions to Journal of Anatomy, Anatomical Record, etc.....	2203.50
Total expenditures.....	2484.10
Balance on hand	\$ 211.59
Balance on hand, deposited in the name of the American Association of Anatomists in the Corn Exchange Bank, New York City.	

On motion the report of the Auditing Committee and the Treasurer were accepted and adopted.

The Committee on Nominations through its Chairman, Professor Thomas G. Lee, placed before the Association the following names: For members of the Executive Committee, term expiring 1922, Professors C. W. M. Poynter, and H. M. Evans.

On motion the Secretary was instructed to cast a ballot for the election of the above named.

The Secretary presented the following names recommended by the Executive Committee for election to membership in the American Association of Anatomists:

- BAKER, WILMER, M.D., Assistant Professor of Anatomy, University of Virginia, *University, Virginia.*
- BECK, CLAUDE S., A.B., Medical Student, *Johns Hopkins Medical School, Baltimore, Maryland.*
- DAVIS, CARL L., M.D., Professor of Anatomy, *George Washington University, Washington, D. C.*
- DAWSON, ALDEN B., A.M., Ph.D., Assistant Professor of Microscopical Anatomy, *Loyola University Medical School, 706 S. Lincoln St., Chicago, Ill.*
- FORD, FRANCIS C., A.B., M.D., Professor of Anatomy, *Hahnemann Medical College and Hospital of Chicago, 2811 Cottage Grove Avenue, Chicago, Ill.*
- FRASSETTO, FABIO, M.D., Ph.D., Director Anthropological Institute, *University of Bologna, Bologna, Italy.* (Present address *Royal Italian Embassy, Washington, D. C.*)
- FRENCH, H. E., M.S., M.D., Professor of Anatomy and Dean of the School of Medicine, *University of North Dakota, Grand Forks, North Dakota.*
- GOULD, HARLEY NATHAN, A.M., Ph.D., Assistant Professor of Anatomy, *School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*
- HOWDEN, ROBERT, M.A., M.B., C.M., D.Sc., Professor of Anatomy, University of Durham, *14 Burdon Terrace, Newcastle-upon-Tyne, England.*
- HOWLAND, RUTH B., Ph.B., Ph.M., Professor of Biology, *Sweet Briar College, Sweet Briar, Virginia.*
- JOB, THESLE T., M.S., Ph.D., Assistant Professor of Anatomy, *Loyola University School of Medicine, 706 S. Lincoln St., Chicago, Ill.*
- KRAUSE, ALLEN KRAMER, A.M., M.D., Associate Professor of Medicine, *Johns Hopkins University, Johns Hopkins Hospital, Baltimore, Md.*
- LARSELL, OLAF, Ph.D., Assistant Professor of Anatomy, *University of Wisconsin, Madison, Wisconsin.*
- MACCREADY, PAUL B., B.S., Medical Student, *Johns Hopkins Medical School, Baltimore, Md.*
- MCINTOSH, WILLIAM, A.B., A.M., Student of Medicine, *Johns Hopkins Medical School, Baltimore, Maryland.*
- MARSHALL, MATTHEW, B.S., Assistant in Anatomy, *School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*
- MATSUMOTO, TAKASABURE, M.D., Professor of Anatomy and Neurology, *Chiba Medical College, Chiba, Japan.*
- MEAD, HAROLD TUPPER, B.A., M.S., Associate Professor of Zoölogy, *Tulane University, New Orleans, La.*
- NOBACK, GUSTAVE J., B.S., Instructor in Anatomy, *Anatomical Institute, University of Minnesota, Minneapolis, Minn.*
- PRACHER, JOHN, M.D., Assistant Professor of Anatomy, *Georgetown Medical School, Washington, D. C.*
- ROY, CHARLES K., A.B., M.D., Assistant in Anatomy, *Anatomical Institute, University of Minnesota, Minneapolis, Minn.*
- SHANER, RALPH FAUST, Ph.B., Teaching Fellow, *Department of Anatomy, Harvard Medical School, Boston, Mass.*
- SHIMIDZU, YOSHITAKA, M.D., Professor of Gynecology, *Aichi Medical College, Nagoya, Japan.*

STEWART, FRED WALDORF, A.B., Instructor in Anatomy, *Cornell University Medical College, Ithaca, N. Y.*

SWETT, FRANCIS HUNTINGTON, A.M., Medical Department, U. S. Army, *Norway, Maine.*

TAKENOUCHI, MATSUZIRO, M.D., Assistant Professor of Bacteriology and Immunology, *Medical College, Imperial University of Tokio, Tokio, Japan.*

VANCE, HARRY WELLINGTON, A.B., Medical Student, *Johns Hopkins Medical School, Baltimore, Md.*

WEGEFORTH, PAUL, A.B., M.D., Captain M. C., U. S. A., *306 Grangu Bldg., San Diego, Calif.*

On motion the Secretary was instructed to cast a ballot for all the candidates proposed by the Executive Committee. Carried.

The special committee on the nomenclature of the sympathetic nervous system reported progress and was retained.

The Secretary read to the Association communications from the Anatomical Society of Great Britain and Ireland regarding a revision of the present anatomical nomenclature and requesting that the American Association of Anatomists appoint a committee with power to take up the matter of collaboration in revising the existing anatomical nomenclatures.

The question of adjustment and modification of nomenclature was extensively discussed by Professors Huntington, McMurrich, Bensley, Terry, Senior, Todd and Jackson. It was finally moved by Professor B. D. Myers, seconded and carried that the Chair appoint a committee to draft resolutions regarding the revision of anatomical nomenclature; and that such a committee be instructed to report back to the Association on the last day of the present session.

The Chair named on this Committee Professor J. Playfair McMurrich, Chairman, and Professors George S. Huntington and H. H. Donaldson.

Through the Secretary the Executive Committee requested the Association to instruct them regarding the proper interpretation of the clause from the constitution relating to the election of new members; Article V, Section 1.

The discussion was based on the proper administration of the clause "Candidates for membership must be persons engaged in the investigation of Anatomical or cognate sciences." The

principles involved in this question were discussed by Professors W. H. Lewis, Evans, Retzer, Emmel, McMurrich, Huntington, Bardeen and Donaldson.

Finally it was moved by Professor Bardeen, seconded and carried:

That it is the sense of the members of this Association that the Executive Committee should as a rule interpret the existing statutes on requirement for membership to mean that the candidate shall have published one or more contributions to anatomy or cognate sciences.

The Secretary then presented for the consideration of the Association the two circular letters recently mailed to the members in reference to the future arrangements for publication of the American Journal of Anatomy and The Anatomical Record. The first of these circular letters was drawn by the Trustees of the Minot Memorial Fund in whom is vested the title of the two above named journals.

The Trustees presented two proposals; in the first place to transfer the title of the two journals to the American Association of Anatomists, and to allow the Association absolute control and all financial responsibility for the organization and conduct of the two journals. In the second plan the title of the two publications was to be transferred to the Wistar Institute of Anatomy and Biology, and the journals were to be conducted under a Board of Control consisting of the trustees of the Minot Memorial Fund and members elected by the American Association of Anatomists.

In connection with these proposals from the trustees of the Minot Memorial Fund the Director of the Wistar Institute, Dr. M. J. Greenman, had drawn the second circular letter to show in general the growth and financial condition of the journals under the present arrangements for publication by the Wistar Institute.

The great importance of the proposals presented in these circular letters was appreciated by the Association and very fully discussed by Director Greenman and Professors Huntington, Stockard, Coghill, McMurrich, Knowler, and Bardeen.

Finally it was moved by Professor Bardeen, seconded by Professor Jackson and carried.

That, *whereas* the members of this Association are themselves primarily concerned with the publication and support of anatomical journals in this country; and, *whereas* information concerning the past, present and possible future, status of the existing journals in this field, the American Journal of Anatomy and the Anatomical Record is incomplete.

Be it *resolved* that the President of the Association be requested to appoint a committee of three to report at the next annual meeting of the Association possible plans for the future publication of these journals together with such other recommendations as may seem desirable as a result of their investigation.

The President appointed such an investigation committee consisting of Professor George L. Streeter, Chairman; and Professors Charles R. Bardeen and Clarence M. Jackson.

On motion the business session adjourned.

SATURDAY, APRIL 19. A BUSINESS SESSION FOLLOWED THE MORNING SCIENTIFIC SESSION.

It was announced by the Secretary that the Executive Committee had voted to hold the *next annual meeting of the Association in Washington, D. C., during the week preceding Easter Sunday, 1920.*

The committee appointed at the previous business session to draft resolutions regarding the revision of anatomical nomenclature reported through its Chairman, Professor J. P. McMurrich, as follows:

Whereas this Association is fully persuaded that both from the standpoints of instruction and investigation a uniform and definite terminology is essential for the progress of Anatomy and *whereas* the Basal Nomenclature furnishes a basis for such a terminology and has been employed very generally in the Medical Schools of this country and in the text-books used by our students.

Resolved that this Association considers it inadvisable to abandon the Basal Anatomical Nomenclature and recommends that a committee be appointed from the Association to confer with the Anatomical Society of Great Britain and Ireland with a view to cooperating in a revision of that nomenclature.

The report was accepted, and it was moved and carried that President Bensley appoint such a committee with himself as Chairman.

The following resolution was introduced by Professor F. T. Lewis and seconded by Professor Bardeen in recognition of the valuable aid and stimulation that has been rendered to science in this country through the publications edited by Professor J. McKeen Cattell.

The American Association of Anatomists express to Professor J. McKeen Cattell its grateful appreciation of the ability and unfailing devotion to scientific progress shown in his editorship of "Science" and other scientific journals, which, while serving their broader purposes, have been so often of direct benefit to anatomists.

The resolution was passed by the Association and the Secretary was instructed to address a copy of the same to Professor Cattell.

It was moved by Professor Bardeen and voted that the Association express through the Secretary its thanks and sincere appreciation of the cordial hospitality and splendid manner in which the Association has been accommodated and entertained by the Medical School of the University of Pittsburgh. In particular the thanks and appreciation of the Association are expressed to Professor Retzer and his associates in the Department of Anatomy.

The meeting was then adjourned.

CHARLES R. STOCKARD,

Secretary of the Thirty-Fifth Session of the American Association of Anatomists.

ABSTRACTS OF PAPERS
PRESENTED AT THE
THIRTY-FIFTH SESSION
OF
THE AMERICAN ASSOCIATION OF ANATOMISTS
APRIL 17, 18 AND 19, 1919

ABSTRACTS OF PAPERS

1. *On the phagocytic capacity of the splenocytes of the rabbit.* WILLIAM H. F. ADDISON, University of Pennsylvania.

The phagocytic capacity of the splenocytes of the rabbit's spleen was studied after the following experimental procedure: Washed pigeon's blood corpuscles were injected into the circulation of the rabbit. The usual hemolysis of the pigeon's corpuscles ensued, and, as an almost immediate effect in a certain number of cases, was followed by the release of great numbers of mature and immature blood-cells from the bone-marrow. Both the hemolyzing blood of the pigeon and the bone-marrow cells of the rabbit, being brought by the circulating blood to the spleen, were delayed within the cavernous blood channels of the pulp, and were there exposed to the phagocytic action of the splenic cells. Of the products of hemolysis of the pigeon blood, after a single injection, comparatively little was retained in a visible form within the cells of the spleen. By using microchemical tests, however, an increased amount of iron-containing substances were demonstrable in the splenocytes, and to a much less extent and in some cases not at all, in the endothelial and reticular cells. Towards the bone-marrow cells, however, the reaction was quite striking. By the end of six hours after injection the bone-marrow cells, notably myelocytes and polymorphonuclears, had been caught in large numbers within the spleen, and soon these began to be taken up by the splenocytes. This process continued so that at sixteen hours the splenocytes were conspicuous by their large size. Some attained lengths of 25 to 30 to 50 micra, and these larger ones contained as many as ten to twenty ingested cells, when viewed in a $4\ \mu$ section. In this special rapidly induced, but non-infectious experimental condition, where cells and cell fragments are the stimulus to phagocytosis, the splenocytes are the first to act, and continue to act as the main phagocytic agents.

2. *The relation of the pituitary and thyroid glands of Bufo and Rana to iodine and metamorphosis.* BENNET M. ALLEN, University of Kansas.

Administration of iodine mixed with flour brings about precocious metamorphosis in Bufo tadpoles from which the pituitary gland has been removed. This is accompanied by a marked shrinkage of the body. Iodine has no effect upon the changed color produced by the removal of the pituitary gland. In these operated tadpoles, the absence of the pituitary gland normally results in scanty deposition of colloid in the thyroid gland. Iodine feeding does not cause any marked increase in colloid deposition in the thyroid glands of these pituitaryless tadpoles.

Great progress toward metamorphosis was produced by feeding iodine to Bufo and Rana tadpoles from which both the pituitary and thyroid glands had been removed. There is every evidence that complete metamorphosis would have been attained if the tadpoles had lived.

3. *On the functional relations of the suprarenal gland and the retinal pigment.* LESLIE B. AREY, Northwestern University Medical School.

The influence of extremes of temperature on the position of the visual cells and retinal pigment of dark-adapted anurans differs both in degree and kind from that exhibited in other vertebrates. In the frog these temperature changes are of maximal order—such as has been associated chiefly with light-adaptation. This unusual response may conceivably depend either upon direct nervous control or on hormone activation.

Controlled experimentation proves that adrenalin is able to induce, for example, maximal pigment expansion in the frog. Extracts of other endocrin glands fail to exert a similar influence. On the contrary, certain other observations are suggestive of nervous control.

A method of attack has been devised which aims to test the reality and extent of influence of each type of activation both under normal and experimentally artificial conditions.

4. *On the use of the term 'sympathetic nervous system.'* WAYNE J. ATWELL, University of Buffalo.

The term 'sympathetic nervous system,' if it is to be retained in neurological nomenclature, should be used in the broadest possible sense, denoting that part of the peripheral nervous system which is concerned with the innervation of smooth muscle, cardiac muscle and glands. Both afferent and efferent nerves should be considered as included, and in the efferent system both preganglionic and postganglionic neurones—those which leave in the craniosacral outflow as well as those in the thoracolumbar. This is apparently the sense in which the term is used by Professor Herrick in his *Neurology* and by Prof. Warren H. Lewis in the latest edition of *Gray's Anatomy*. It was so employed by Professor Huber as early as 1897, although he preferred not to include the preganglionic neurones. 'Autonomic nervous system' should be used synonymously with 'sympathetic nervous system,' or at least should be applied to the entire efferent portion of the system. The application of 'sympathetic' and of 'autonomic' to restricted parts of the efferent system, as has been done by Langley and the German school, respectively, is to be deprecated from a morphological viewpoint.

If 'sympathetic' is to be employed in the broad sense indicated it probably will be found desirable to adopt single words to replace the rather awkward compounds 'craniosacral component' and 'thoracolumbar component.' The adoption of such new terms should come only after a conference of anatomist, physiologist, pharmacologist, and clinical neurologist.

5. *Pelvic fascia.* ARLIE RAY BARNES (introduced by B. D. Myers), Indiana University.

One of the greatest sources of confusion in the description of the pelvic fascia has been the attempt to show that it could be traced as a

continuous layer over the entire pelvis, and, by some authors, even over the perineum.

There are certain structural units of the pelvic fascia, units that can be accounted for by differences in development. The coalescence of certain contiguous layers of peritoneum and their subsequent replacement by connective tissue lamina, represents one type of fascia. The umbilicovesical and the rectovesical fascia belong to this first class.

Secondly, we have fasciae related to muscles, for example, the fascia covering the obturator internus, that covering the levator ani, and that of the perineal muscles.

Lastly, we have that very considerable and much misunderstood mass of visceral mesodermal tissue surrounding the branches of the hypogastric arteries on their way to the viscera of the pelvis.

If we keep these three structural units in mind, having as our object the study of their arrangement, the question of continuity will become less important and will take care of itself.

The umbilicovesical fascia. The general shape of the umbilicovesical fascia is triangular with apex at umbilicus and lateral borders becoming continuous with the peritoneum just lateral to the obliterated umbilical arteries. The central portion of the umbilicovesical fascia is very closely adherent to the anterior surface of the bladder where it is very thin and can be demonstrated only in most favorable conditions. It ends by joining the capsule of the prostate gland. Its lateral portions join the mesodermal tissue about the vessels going to the bladder. This fascia is related to the transversalis fascia anteriorly; posteriorly it is separated from the peritoneum by the umbilicovesical sheath.

The umbilicovesical sheath is the remains of mesodermal connective tissue which originally surrounded the allantois and umbilical arteries. It therefore stretches from one obliterated umbilical artery to the other, enclosing in its upper median portion the ligamentum umbilicale medium, and in its lower central portion, the bladder. For the bladder it forms a closely adherent sheath, incorrectly called visceral pelvic fascia by Cunningham. The sheath has the shape and same general limits as the umbilicovesical fascia, with which it blends over the anterior part of the bladder.

Rectovesical fascia. The rectovesical fascia, of peritoneal origin, joins the peritoneum where it leaves the bladder to be reflected onto the anterior surface of the rectum. It extends downward between the rectum posteriorly and the seminal vesicles and bladder anteriorly to be attached to the capsule of the prostate gland. Occasionally an offshoot of this fascia passes down between the seminal vesicles and bladder to be attached to the capsule of the prostate gland. Laterally, this fascia is lost as it blends with mesodermal tissue about the vessels to the bladder.

The obturator and levator ani fasciae. The iliac fascia passes over the brim of the pelvis minor to become the pelvic fascia. It con-

tributes the thin fascial coat on the medial aspect of the obturator internus. This continues downward to be joined by the fascia lunata as mentioned above.

The levator ani is very variable in its origin and its fascial coverings vary also.

Two terms need definition: The 'white line' or arcus tendineus fasciae pelvis stretches from the back of the lower portion of the symphysis pubis to the ischial spine. It constitutes, both the lateral and anterior true ligaments of the bladder. The arcus tendineus levatoris ani has posterior attachment to the spine of the ischium, but its anterior attachment is to the superior ramus of pubis anterior to the obturator canal. When present, it helps to furnish origin for the iliococcygeal portion of the levator ani. This latter tendinous arc is not derived from a cleavage in the obturator fascia but may sometimes be adherent to the medial surface of the obturator internus fascia.

The superior surface of the levator ani is always clothed with a division of the pelvic fascia, represented superiorly in its iliococcygeal portion as the aponeurotic remains of the iliococcygeus. Except in the case of very low origin from the 'white line,' the perimysium on the inferior surface is too thin to be dignified by the term fascia.

Connective tissue surrounding branches of hypogastric arteries. The blood-vessels to the bladder are surrounded by mesodermal connective tissue which does not deserve the name of fascia. The rectum is surrounded by a plexus of vessels imbedded in similar tissue, constituting a covering, but again, hardly deserving to be called fascia.

Fascia lunata. The fascia lunata is a sheath for the internal pudendal vessels and nerves. It can be demonstrated about these structures as they leave the pelvis through the greater sciatic foramen and may be traced to the urogenital diaphragm. It has a slight attachment to the sacrospinous ligament, but extensive attachments to the inferomedial border of the sacroptuberous ligament. A portion of the fascia lunata is in relation to the obturator internus. In this connection, attention must be called to a horizontal septum, the lamina terminalis, passing from the superior border of Alcock's canal to the fascia on the lateral wall of the levator ani muscle. This limits the ischio-rectal fossa superiorly. Above this lamina terminalis, bounded laterally by the fascia covering obturator internus and medially by the levator ani, is a space, triangular in coronal section, the supratregmental space. This is usually described as a part of the ischio-rectal fossa. Anteriorly the ischio-rectal fossa ends, at the level of the posterior border of the urogenital diaphragm, in several blind pockets opening posteriorly and filled with fat.

In the urogenital diaphragm, the sheath or fascia lunata is broken up to be prolonged about the branches of the pudendal nerve and artery. Thus, it is a considerable element in the formation of the urogenital diaphragm, especially of the superior layer.

The urogenital diaphragm. This structure is ordinarily considered to consist of a superior and inferior layer enclosing between them the

deep transverse perineal muscle, the sphincter of the membranous urethra, together with branches of the pudendal artery and nerve. The following is a more detailed statement. The inferior layer of the urogenital diaphragm occupies the interval between the posterior border of the transverse ligament of the pelvis and the posterior-superior border of the superficial transverse perineal muscle. The bulbocavernosus, the ischiocavernosus, and the transverse superficial perineal muscles are surrounded by tubular investments derived from Colle's fascia, and on their deep surface their sheaths join the inferior layer.

The superior portion of the urogenital diaphragm is composed of several units. Anteriorly, the first of these is the arcuate ligament. Next comes the transverse ligament of the pelvis, separated from the arcuate ligament by the dorsal vein of the penis. The third element is a considerable septum, 4 to 8 mm. in thickness, deep to the area covered by the inferior layer of the urogenital diaphragm and blends superiorly with the capsule of the prostate gland. The urogenital diaphragm is attached laterally to the superomedial surface of the ischiopubic rami; posteriorly to the sphincter ani by the intervening perineal body. The bulb of the penis rests upon its inferior surface.

6. *The weight of the leg in living men.* ROBERT BENNETT BEAN, University of Virginia.

"The relative weight and dimensions of the leg to the rest of the body in amputations at the thigh and above and below the knee, based on conditions obtaining in the live normal man," were secured at the request of the Red Cross Institute for Crippled and Disabled Men.

The leg of an average-sized cadaver, not emaciated, but with almost no fat, weighed 15.56 per cent more than the water it displaced at 70° F., and the leg of another cadaver, with considerable fat although not extremely obese, weighed 10.94 per cent more than the water it displaced. The middle part of the leg about the knee was relatively heavier compared with the water it displaced than either the thigh or foot.

A tank was made into which about 500 soldiers legs were dipped, and the water displaced was weighed at 70° F., for the three parts of the leg: the foot, the knee, and the thigh. The water displaced by the foot is 1.6 per cent of the body weight, by the knee 4.7 per cent, and by the thigh 6.9 per cent. The weight of the two lower extremities is about 30 per cent of the body weight.

The size of each part is larger in the tall than in the small, and this difference is greatest in the foot, less in the knee and least in the thigh. Those from 20 to 25 years of age have larger feet than those from 25 to 30 years, but this is because more mesophylomorphs were examined between the ages of 20 and 25 and more hyperphylomorphs were examined between the ages of 25 and 30 years—a fortuitous circumstance.

The hyperphylomorph has a long, narrow, slender foot with high arch, and the mesophylomorph has a broad, short, stocky foot with low arch. The foot of the extreme hyperphylomorph displaced 873 cc. of water, the knee 2738 cc., and the thigh 3972 cc.; the foot of the mesophylomorph displaced 1142 cc., the knee 3875 cc., and the thigh 5262 cc.

7. *Some racial characteristics of the spleen weight in man.* ROBERT BENNETT BEAN and WILMER BAKER, University of Virginia.

The material used consists of postmortem records from the Charity Hospital and Touro Infirmary, New Orleans, La., the Johns Hopkins Hospital, Baltimore, Md., and the University of Virginia Hospital, Charlottesville, Va., and the authors wish to thank Doctor Duvall, Doctor Landfried, Doctor MacCallum, and Doctor Marshall for the use of records from their laboratories. The spleens of 1341 white men, 1338 negro men, 441 white women and 554 negro women are utilized in the present study.

The spleen of the negro is smaller than that of the white, and this difference is well marked in both normal and pathological spleens. The white male spleen weighs about 140 grams, the negro male 115 grams, the white female 130 grams and the negro female 80 grams, in the normal adult, although the number of normal spleens is too few to justify this as final.

The racial difference is great in spite of the fact that more tall, young, well-nourished negroes and more small, old, thin whites constitute the records, and the latter difference is especially noticeable between the males. This would seem to indicate that the whites under present environment are more viable than the negroes, and the large size of the spleen in the whites may play a part in this greater viability, especially when we consider that the spleen reacts to infections, and may play a large part in the resistance of the body to disease.

8. *The weights of the human organs (preliminary report).* ROBERT BENNETT BEAN and WILMER BAKER, University of Virginia.

Material: Johns Hopkins Hospital Autopsy Records, twenty-two years up.

Heart. Average weight: White male, 315.4 grams, white female, 265.4 grams, negro male, 325.1 grams, negro female, 262.0 grams. Most heart weights are between 200 and 425 grams in the male and between 150 and 350 grams in the female. Racial differences are slight and sexual differences are probably due mainly to differences in body size.

The heart weight increases with increase of stature, age, and nourishment. Of these nourishment has the most influence, age less, whereas stature has the least influence on the heart weight.

Spleen. Average weight: White male, 184.7 grams, white female, 165.8 grams, negro male, 144.4 grams, negro female, 117.9 grams. Racial differences are very marked, but sexual differences are no greater than should be expected.

Age has very little influence on spleen weight. There is a constant, though small, increase with stature, and a larger increase with nourishment.

Liver. Average weight: White male, 1695.7 grams, white female, 1510.3 grams, negro male, 1670.5 grams, negro female, 1471.9 grams. Racial differences are small and sexual differences are about the same as in other organs.

There is a steady increase in liver weight with stature, and a more marked change relative to nourishment, but the liver weight bears an inverse ratio to age.

Kidneys. Average weight: White male, 351.9 grams, white female, 314.4 grams, negro male, 365.8 grams, negro female, 320.9 grams. The kidneys of the negro are slightly heavier than those of the white, probably because of the better physique of the former.

Kidney weight increases with stature and nourishment, especially the latter. After the age of 40 there appears to be a slight decrease in kidney weight.

9. *The relative distribution of clasmatoocytes in the various organs of the seven-day chick embryo.* CLAUDE S. BECK (introduced by W. H. Lewis), Johns Hopkins Medical School.

Small pieces of tissue from the various organs were placed in neutral red Locke's solution and then were mounted as living spreads. There are striking differences in the number of clasmatoocytes that are present in the different tissues of the chick. In the subcutaneous tissue clasmatoocytes are present in the greatest abundance. Here they lie everywhere in the loose reticulum of the connective-tissue cells. They are absent from the epidermis. In the submucosa of the stomach, intestine, and esophagus clasmatoocytes are present in large numbers, in some places they appear in swarms. In the subserous tissue they are numerous. In the musculature of the gut they lie between the muscle bundles. They are absent in the endothelial lining of the gut. In the cornea, in striped muscle, in the pia arachnoid clasmatoocytes are not so plentiful as in the preceding structures, but they are present in no small numbers. In the amnion and in the sclera there are few clasmatoocytes. The mesonephros and the metanephros contain few clasmatoocytes, but they are abundant in the walls of the Wolffian duct. In the liver clasmatoocytes are present in very small numbers. In the optic lobes of the brain and in the retina no clasmatoocytes could be found. The method was unsatisfactory for the examination of the spinal cord and parts of the brain. Clasmatoocytes seem to be present in the region of the choroid plexus and in the telencephalon medium. They are absent in the choroid coat of the eye.

10. *The digestive tract of the five-day chick.* (Lantern.) E. A. BOYDEN, Harvard Medical School.

This study is based upon a new and large model of the entire digestive tube of the five-day chick. In comparison with mammalian

embryos, several interesting features are brought out, notably concerning the pancreas and its islands and accessory intestinal diverticula. But since these are not readily described in abstract, the following account is limited to a discussion of retrograde changes in the epithelium of the pharynx. In a former paper on vestigial gill filaments in birds and reptiles (Am. Jour. Anat., '18) the writer described the presence of conspicuous 'degeneration vesicles,' 10 to 20 micra in diameter, in the branchial epithelium of the bird throughout the period of gill formation, that is, from the beginning of the fourth to the middle of the eighth day of incubation. These were described as accompanying an activity of the branchial epithelium of which the filaments seem to be the fruition. Further study has convinced me that these vesicles are distended phagocytes comparable to those found in certain pathological lesions of the human adult. Indeed, Professor Mallory, to whom they were shown, had no hesitation in describing them as endothelial phagocytes. The number of these and the extent to which they are gorged with broken-down epithelial cells, one or two dozen inclusions often appearing in a single leucocyte, indicates a very active process of resorption which is removing the proliferating epithelium, but not fast enough to prevent the growth of filaments. Since these observations were made, a similar process, though somewhat less active, has been detected in the optic stalk and much less frequently in the outer layer of the lens and the pigmented layer of the retina. Perhaps the most striking feature is the very early period in which this resorption takes place, at a time when the primitive blood cells exhibit so little differentiation. I have been able to verify their appearance in large numbers in the branchial epithelium of chicks of 2 days and 21 hours, at which time these leucocytes contain but few ingested epithelial cells and resemble the phagocytic lymphocytes figured by Danchakoff ('08 a). She found them within the blood-vessels of the area vasculosa of an 18-somite chick (40 hours), and in my specimens similar cells occur in small numbers in the capillaries and vessels adjacent to the branchial epithelium. Those of the circulating blood may well be the source of the phagocytes in question. It is possible, however, that they may be derived from the mesenchyma. In that case there must be an earlier differentiation of interepithelial tissue than is described by Doctor Danchakoff ('08 b), who finds that even to the fourth or fifth day the mesenchymal cells are wholly undifferentiated, quite alike and equipotential. This accords with an origin by migration from the vessels, but the evidence is not conclusive.

11. *On the interaction of primary femoral ossification, thigh muscular differentiation, knee and hip-joint formation, during the period of rotation of the hind limb of the pig (Sus scrofa).* EBEN J. CAREY, Creighton Medical College.

It has been shown previously (Anat. Rec., vol. 11, no. 6, 1917, and vol. 14, no. 1, 1918) that the earliest bone formation in the femur coincides in time with the manifestation of contractility of the thigh mus-

culature and in site with the region of greatest mechanical tensile stress incidental to the rotation of the limb and bending of the femur. From direct observation, it seems probable that there is a definite action of the developing parts of the thigh upon one another resulting in dependent differentiation. This incipient differentiation, however, is not wholly dependent, since the structure of the reacting, as well as that of the stimulating part is contributory to the quality of the effect.

The most rapidly growing part of the thigh is the skeletal core. This blastemal skeletal zone of growth appears to exert a traction force upon the contiguous syncytial mesenchyme. This mesenchyme is first condensed as premuscular tissue with its nuclear long axis in the direction of skeletal growth. The cytoplasm of the premuscular mass becomes drawn out and subsequently the myofibrils appear as elongated strands, also, directed along the lines of skeletal growth. As the myofibrils differentiate and functionate in embryos of 15 to 20 mm. in length, rotation of the hind limb is begun. There is also a tendency on the part of the myofibrils to restrict the longitudinal growth of the cartilaginous skeletal core. The femoral rod, which is outlined by a condensation at the future knee and hip joints, by this time, is consequently bent. The weakest part of the rod is at the middle on its convex aspect. It is here that eventually the first steps in fibrogenesis of the periosteum, degeneration of the cartilage cells and genesis of primary bone appears in embryos 25 to 32 mm. in length.

The hip-joint cavity appears in embryos 19 to 24 mm. in length. This cavity formation coincides with the first adducting action in the rotation of the hind limb. Likewise, the formation of the retropatellar cavity at the knee, which appears in embryos 25 to 30 mm. in length, coincides in time or shortly follows flexion of the knee joint.

From the order of appearance of the differentiating blastemal skeletal core, myofibrils, cavity formation of the knee and hip joints, and primary femoral ossification there appears to be a definite interaction of the developing parts on one another, during the rotation of the hind limb which points to dependent differentiation and not purely self-differentiation.

12. On the development of the lymphatics in the stomach of the embryo pig.

JAMES R. CASH, Johns Hopkins University.

In this study the lymphatics of the stomach were followed by the method of injection. In living embryos measuring from 30 to 60 mm. the injection was made through the retroperitoneal sac. In embryos measuring from 80 to 150 mm. injection was made directly into the stomach wall at the lesser curvature. In the embryo pig the retroperitoneal sac is large and its anterior end lies opposite the coeliac axis.

The lymphatics of the stomach arise from the anterior end of the retroperitoneal sac by two main trunks. Thus, in their origin the gastric lymphatics are analogous to those of the intestine which arise by two forks, right and left, and form an arch around the wall.

The right gastric vessels pass up behind the stomach and invade it at three points: 1) at the esophagus by numerous vessels which form a very dense periesophageal ring; 2) at the lesser curvature by a great mass of vessels which pass directly to the stomach wall; 3) at the pylorus by one or two vessels.

The left gastric trunk (splenic trunk) divides into two branches, One passes anteriorly directly to the cardiac pouch. The other passes through the splenic ligament, to the hilum of the spleen, then traverses the gastrosplenic ligament to the center of the greater curvature of the stomach, along which it ramifies to right and left. These lymphatics then anastomose, both over the anterior and posterior walls, with those from the lesser curvature where connections are formed with the lymphatics of the esophagus and duodenum.

13. *Reaction of cells in tissue culture to ether.* JAMES R. CASH, Johns Hopkins Medical School.

Connective-tissue cells, muscle buds, and nerve fibers from explants of embryonic chick tissue of former Lewis solution were studied under influence of ether vapor. Within one to three minutes numerous definite, clear, homogeneous vesicles bulge out at points on surface of cell. Many of these rapidly change shape, assuming protean forms.

Following sublethal amounts of ether vapor, the vesicles flow back and the cell assumes normal appearance. After slow ether death the vesicles remain active; but when rapid death ensues, the entire cell assumes a rounded form, and few, if any, vesicles appear.

There are concomitant changes in the nucleus, nucleolus, mitochondria, and other cell granules.

Similar changes are readily produced by subjecting old cultures (three days) to markedly hypotonic salt solution. Immediately (within 30 seconds) numerous vesicles appear, change shape characteristically for a short time, and flow back into the cell. Such changes are less readily produced in young, healthy cells. Similar vesicles have occasionally been noted in degenerating cultures (four to five days).

From such observations it would seem that these vesicles are evidence of degeneration by which changes at points in the cell membrane occur, allowing rapid inhibition of water. In the functionally active cell this change is probably overcome by the internal metabolism of the cell.

These vesicles are different from the pseudopodia described in either the resting or the dividing cells.

14. *Mesenchyme and its biological properties.* V. DANCHAKOFF, Columbia University.

It has been shown lately that only under typical conditions does the greatest part of the embryonic mesenchyme become the anlage for the interstitial tissue of various organs. It may, however, under other conditions differentiate into products which though encountered in a normal organism do not enter normally into the constitution of a definite

organ. As seen from the lantern slides and microscopical preparations, the embryonic mesenchyme of various organs may greatly proliferate under definite experimental conditions and develop into large accumulations of granuloblastic tissue.

In the normal development the embryonic mesenchyme transforms in most of the organs into 'interstitial connective tissue.' No special function has been ascribed to this tissue besides the holding together of the specific elements characteristic of various organs. It is true that a phagocytic activity has been often described as belonging to the cells of the interstitial tissue.

New data concerning the remarkable relationship developing in a culture on the chick allantois between adult splenic mesenchyme and proliferating cells of the Ehrlich sarcoma seem to point out the fact that the phagocytic and digestive power of the adult mesenchyme may be an important factor in the phenomenon of the immunity observed through the animal kingdom.

As seen from the lantern slides, tumor cells either in the resting period or in mitosis are surrounded by mobilized cells of the splenic mesenchyme and submitted to a gradual complete digestion. This phenomenon is not altogether different from the ingestion of erythrocytes of blocks of disintegrating tissue by a mobilized mesenchymal cell, frequently observed in the organism. Only in this case the ingestion is effected by a group of mesenchymal cells.

The fate of the ingested cells in both cases is identical; they are digested in the first case intracellularly; in the latter, intraplasmodically. If I may say so, the phagocytic and digestive activity of a mesenchymal cell is usually directed against dead particles and possibly weakened cells of its own kind. In the case of heteroplastic tissue, however, mesenchymal cells may ingest a living cell and submit it to a complete digestion. The biological properties of the mesenchyme, i.e., its digestive power, must certainly be taken into consideration in our attempt at solving the problem of the resistance of the organism against heteroplastic grafting.

15. *An experimental test of the possibility of differential selection of germ cells (in the fowl).* C. H. DANFORTH, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE.

It is commonly assumed that heritable differences in adult structures are due to more or less specific determiners thought to be present in the germ cells. If this seemingly well-founded assumption is valid, it follows that the germ cells produced by a heterozygous animal must fall into several classes, and the question naturally arises as to whether such germ cells may not react differently to chemically or physically changed surroundings, or at least show somewhat different potentialities in their competition with each other. In order to test this question, cocks heterozygous in regard to brachydactyly, polydactyly, color, and shape of comb were mated to hens homozygous for these traits in their recessive forms and a record made of the numbers in each

class of young produced before and after treating the males with alcohol. The data thus obtained indicate that the administration of alcohol by Stockard's inhalation method alters the proportion of certain classes of chicks produced. This is interpreted to mean that (contrary to Pearl's opinion) a mildly toxic agent may select between germ cells on the basis of the Mendelian determiners which they carry. Incidentally, it may be observed that a recognition of the fact that the probability that a germ cell will function is in some degree dependent upon the determiners which it carries, may lead to a satisfactory explanation for orthogenesis.

16. *A study of coagulation in embryonic blood.* V. E. EMMEL, University of Illinois, College of Medicine.

In the course of an experimental study of the origin of the non-nucleated erythrocytes or erythroplastids (Emmel, '14) certain striking differences were observed in the coagulation of embryonic blood. In the present investigation, undertaken in coöperation with two of my students, Doctors Fish and Levinson, a more extended study has been made of the subject. It is the purpose of the present report to present certain results attained in experiments made on the blood of pig embryos at various stages of development.

17. *On the segregation of macrophage and fibroblast cells by means of vital acid dyes and on the cause of the differential effect of these substances.* HERBERT McLEAN EVANS AND KATHARINE J. SCOTT, University of California.

The two great cell strains of the connective tissue of mammals—the fibroblast and the macrophage cells—exhibit a pronounced and characteristic difference in their reaction to intravital acid dyes. This difference shows itself in the appearance of two sharply separated types of response to the dye—in the size, form, and number of the vital dye 'granules'—and the fact that these types of vital dye response are associated with those other characteristics which permit us to designate fibroblast and macrophage cells. The mitochondrial apparatus of the connective-tissue cells cannot be said to be electively stained by means of the vital acid dyes. The vital dye 'granules' in the case of both fibroblast and macrophage cells are neither chemical combinations of the dye with the protoplasm nor physical tinging of preexisting cell organs, but are actual accumulations within the cell of the vital dyestuff employed in fluid, high colloidal, flocculated, or crystalline form. The number and size of the dye 'granules' within the cell in the case of any one dye are dependent on the concentration and time of dye dosage, and in the case of various dyes, follow a similar law except that there occur characteristic differences in the structures created by various dyes when a standard dosage is employed. In the case of several dyes, among several hundred which were tested, there occur color changes in the dye substance in accordance with whether it is in solution or in solid or semisolid form. This

metachromasia has been carefully investigated by Werner Schulemann and one of us. It would appear to prove conclusively that the dye is at first in soluble state in its place of deposit within the cell, but that it undergoes concentration so as to be thrown out of solution, the solid form, whether amorphous or crystalline, showing a different color from the fluid. By certain dosages with some of the numerous dyes of this series, a true crystallization of the vital dye can be made to occur within the living cell. The employment of acid dye of marked color difference, but of similar physical state, produces vital dye 'granules' of mixed color; the employment of differently colored dyes of markedly different physical state, produces vital dye structures in which only a partial color contamination occurs. All of these phenomena are exhibited by the dye deposits of both macrophage and fibroblast cells. The ingestion of these dyestuffs is usually associated with their separation from the living protoplasm by virtue of a segregating power of the cell; for such segregation, granules, minute vesicles and vacuoles of many sizes may be created in addition to those already present in the cell. We have called the ensemble of these structures the 'vacuolar apparatus' of the cell. The vacuolar apparatus is electively stained by neutral red and by certain other basic dyestuffs when these are applied supravitaly. In the case of the fibroblast cells, but never in the macrophages, the vacuolar apparatus shows an interesting tendency to exhibit peculiar filar modifications so that elaborate thread structures may result in areas where the dye application has been particularly intense. With the exception of this peculiar or qualitative difference, the usual difference in the vital staining reaction of macrophage and fibroblast cells is to be interpreted as a quantitative one only, and the nature of the dye 'granules' in both cells is identical. The quantitative difference in reaction to the vital dyes is, however, a remarkably sharp histophysiological test. The difference in the behavior of the two cell strains is shown both by their unequal power to store and to liberate their protoplasmic deposits of these substances. The power to store these vital dyestuffs is on the part of the macrophage greatly in excess of a similar capacity shown by the fibroblast cell. The macrophage vital dye deposits are, conversely, more susceptible of decolorization and less permanent than are the more minute deposits in the fibroblast cell.

The detailed cytological evidence here summarized will be found in a contribution to the Carnegie Memorial Volume in honor of Prof. Franklin P. Mall and the correlative data on vital dyestuffs of the acid azo series, in a monographic summary by one of us in conjunction with Schulemann and Wilborn which has lain ready for publication for some time.

18. *Studies on sex in the hermaphrodite mollusc Crepidula plana. III. Transference of the male-producing stimulus through sea-water.* HARLEY N. GOULD, University of Pittsburgh.

The gastropod mollusc *Crepidula plana* passes through a male phase, a transitional phase, and a female phase during its life. The male phase

is unstable and occurs only as the result of a stimulus furnished by an individual of the same species larger than the one stimulated.

Complete isolation of small sexually undeveloped specimens over long periods shows that no development of male characters takes place under such conditions further than the formation of a few spermatogonia. In time female characters appear. Small sexually undeveloped individuals confined at fixed distances of from 4 to 7 mm. from large females, where contact is prevented, will in a majority of cases develop male characters to various degrees of maturity. Fewer and less well-developed males are produced under such conditions than when the small animals are nearer the source of stimulus.

Large individuals of *Crepidula fornicata*, a species related to *C. plana*, have not been found to induce male development in small *Crepidula plana* except in a few doubtful cases.

The stimulus to male development acts in such a manner as to indicate that it is a substance given off from the bodies of the large *Crepidula plana*, diffusible through sea-water, but very unstable.

19. *War deafness and its prevention: cochlear observations.* STACY R. GUILD, University of Michigan.

At Minneapolis were reported new methods of testing ear protectors. Of the results of the animal tests the conditions of the middle ears only could be reported then. The present report is of the conditions in 92 cochleae, these being from control and 'protected' ears. Technique: injection fixation with Zenker-formalin; celloidin embedding; decalcification; 'double' embedding in paraffin; 7 μ sections; Heidenhain's hematoxylin and benzopurpurin. Definite lesions to the organ of Corti range from the loss of occasional outer hair cells to almost complete disintegration of the epithelial parts. For purposes of tabulation, the lesions have been designated first-, second-, third-, and fourth-degree injuries; their distribution is shown by charts. The division into more and less efficient groups of protectors is not as sharply marked by the cochlear conditions as by the middle-ear conditions and by the tambour tests; each of the protective measures failed to prevent definite cochlear lesions in one or more of the ears with which it was used. Even here, however, a ranking of the devices is evident. Both from the standpoint of the tests and of special military requirements for field use by troops, the 'Tommy' appears to be definitely the best of those tested. The distribution of the cochlear lesions is also of interest in connection with the physiology of hearing. Four reports of this work, submitted to the National Research Council, have been published in the *Journal of Laboratory and Clinical Medicine*, September, 1917; January, 1918; March, 1918, and January, 1919. (Lantern.)

20. *The anatomy of the 7-mm. opossum embryo.* CHESTER H. HEUSER. The Wistar Institute of Anatomy.

As a part of the plan to work out the development of the systems of organs in the opossum, the anatomy of a stage similar to the 12-mm.

pig is being especially studied. The illustrations are based largely on a series of dissections from three embryos. In one the cerebral and spinal nerves were uncovered and the trunks traced to include the fine terminal branches. Wax reconstructions were made of the pharynx, digestive and respiratory systems, heart, and the left jugular lymph sac. I have also made vascular injections in the living embryos. Fifth aortic arches are present.

The third pharyngeal pouch derivatives have become separated from the pharynx. The ganglion nodosum is fused with the cervical sinus, and there arises from the ganglion a cord of cells which, with a strand from the thymus, makes another connection with the sinus. The hypoglossus is embedded in the nodosum, but does not touch the thymus. The superior laryngeal nerve is distinct: it extends from the mesial border of the cord above referred to and runs between the thymus and the parathyroid.

The jugular lymph sac has become transformed into a much-divided structure with smaller and larger spaces. In an injected embryo of the same litter the lymph sac received no ink, although the injection of the blood vessels is complete.

21. *Experiments with the thyroid, hypophysis and pineal glands of Rana sylvatica.* E. R. HOSKINS and M. M. HOSKINS, University of Pittsburgh.

A. Thyroid. In 105 young larvae the thyroid anlage was cut to pieces, but left in situ, and an additional thyroid anlage transplanted into the animal. Some of the larvae developed accessory thyroids, but this had no effect except perhaps in a few larvae that metamorphosed while small. The time of metamorphosis was not hastened.

B. Hypophysis. The hypophyseal anlage was removed from 116 young larvae after the method of Smith and of Allen, with the usual results in most cases. A few larvae became black. Some of these black larvae metamorphosed, but one did not, although it grew much larger than normal.

Transplantation experiments of the hypophyseal anlage into 62 young larvae gave negative results, although some of the transplants grew.

C. Pineal. The pineal was removed from 70 young larvae, but regenerated either partially or completely and the larvae grew normally. The anlage of the pineal was transplanted into 19 young larvae. It failed to grow.

D. Some of the larvae of the thyroid and hypophysis operations developed small accessory mouth-parts. These were mostly ectodermal outpouchings, but in some of them muscle fibers developed. One of these opened into the oral cavity.

22. *Observations on thyroidless Rana sylvatica larvae kept through the second season of normal metamorphosis.* E. R. HOSKINS and M. M. HOSKINS, University of Pittsburgh.

The larvae nearly reached their maximum size the first summer (66 mm.), but grew again slightly during the winter and more during the second spring and summer (72 mm.). They became relatively long-tailed, the legs grew $\frac{1}{2}$ mm., the head and back flattened, and the eyes became relatively far apart.

The brain acquired a shape practically mature, but at a size much larger than normal, and the liver became nearly mature in shape. The hypophysis became relatively very large, especially the inferior lobe, and this lobe showed an increase in the relative number of eosinophilic cells. The anterior and superior lobes showed little structural differentiation. The thymus glands persisted and became relatively and actually large. They retained the larval shape and structure. The epitheloid bodies (parathyroids) became relatively large. The spleen became large, but was roughly proportional to the size of the larva. The kidneys enlarged both actually and relatively. The internal gills persisted and the lungs became large and functional. The intestines grew long and remained larval in type, as noted by Allen. The ovaries became large and large oocytes developed. Maturation was not seen and oviducts did not develop, so the animals were not sexually mature. The testes became mature and formed spermatozoa which escaped into the kidneys.

By successive extirpations of the end of its tail, a larva was made to regenerate 38.5 mm. of tail. It regenerated one small hind leg once, but not a second time after the regenerated leg was removed. The amount of time required for regeneration of the tail gradually increased.

A larva placed in a moist chamber lived two days, its volume decreased 18 per cent, its tail shrank 24 per cent, and its intestine became strongly contracted to about half the normal size, but did not shorten perceptibly.

23. *An analysis of the theories of pulmonary evolution in the Mammalia.* GEO. S. HUNTINGTON, Columbia University.

The phylogeny of the mammalian lung is considered with especial reference to the development of the extant bronchial architectonic types and their evolutionary significance. The conclusions lead to a revision of the prevalent views of intrapulmonary organization. The paper discusses:

1. The reduction theory of Aeby ('80) and D'Hardiviller ('97) in which the modern problem of bronchial interpretation found its beginning.

2. The theory of the migration of bronchial components, propounded by Willach ('88) and Narath ('92, '96, '01).

3. The summary of the results reached by the writer constituting what can briefly be defined as the selective theory of mammalian pulmonary specialization.

24. *The effects of inanition in the young upon the ultimate size of the body and of the various organs in the albino rat.* C. M. JACKSON and C.

A. STEWART, University of Minnesota.

Thirty-eight litters were used; 113 rats survived, 35 male and 35 female test rats, 27 male and 16 female controls. Groups were underfed from birth to three, six, and ten weeks of age, and from three weeks to twenty weeks or to nearly one year. There upon the test rats were fully refed. They grew variably, but remained permanently stunted, failing to reach the adult size of the controls. Stewart ('16) found perfect recovery after underfeeding from three to ten weeks of age. The ultimate effect, therefore, varies according to the age of the animal and the extent of the underfeeding period. This is in agreement with the results of Aron and Brüning, but disagrees with Osborne and Mendel.

Forty-five of our rats (28 test and 17 controls) were autopsied. The organs in the test animals were compared with the normal at corresponding body weight. Body length and tail length appear slightly subnormal; head, limbs, and trunk nearly normal in weight; skeleton, integument, and musculature usually slightly subnormal, visceral group slightly above normal, and 'remainder' variable.

Of the individual organs, the brain, spinal cord, hypophysis, and lungs average slightly subnormal; the ovaries distinctly so. The heart and alimentary tract are slightly, and the testes and epididymides definitely, above normal weight.

While some abnormalities thus appear, they are usually slight, and in general the organs and parts are nearly normally proportioned in the permanently stunted rats. Thus early starvation apparently retards the later growth process of the body as a whole.

25. *Reversal of striation in contracting muscle.* H. E. JORDAN, University of Virginia.

Merkel first ('72) recorded the phenomenon of stripe reversal in contracting muscle. The investigations of Rollet ('85) and Tournoux ('92) support Merkel's interpretation, and add the concept of a 'contraction band.' The dark band (Q) of uncontracted fibers is bisected by the mesophragma; the dark (contraction) band of contracted fibers is bisected by the telophragma. Merkel and Rollet believe that the anisotropic substance of uncontracted fibers divides along the mesophragma and moves in opposite directions against the telophragmata to form the dark stripes of contracted fibers. Englemann ('73), Van Gehuchten ('86) and Schaefer ('91) dispute the accuracy of such interpretation. Schaefer explains the apparent reversal of striations as an optical effect consequent to the condensation of the J-substance about Z and a relative rarefaction of the Q-substance about M, due to an absorption of the isotropous by the anisotropous constituent. Englemann and Van Gehuchten have apparently proved by means of micropolariscopic studies that the anisotropic substance does not alter its position during contraction, Schaefer, by means of Rollet's gold-

chlorid technic, claims to have established the same fact. A reinvestigation of the phenomenon of stripe inversion in the wing muscle of wasp, by various combinations of fixatives and stains, and studies in hypo-, iso-, and hypertonic solutions, confirms my former conclusion that during contraction there is a true reversal of striations as regards a deeply staining constituent of the Q-disc and the contraction band, and gives the key for the reinterpretation of Schaefer's results in terms of the action of a hypotonic solution.

26. *The sympathetic innervation of the testis in the dog.* ALBERT KUNTZ, St. Louis University School of Medicine.

The sympathetic nerve supply to the testis is derived from the third, fourth fifth, and sixth lumbar segments of the sympathetic trunk. These fibers descend along the course of the spermatic artery and vein. The hypogastric nerve supplies some fibers to the pelvic end of the vas deferens; however, these fibers probably do not reach the testis.

Sympathetic fibers are supplied to all structures in the spermatic cord and testis which contain smooth muscle. There is no evidence that sympathetic fibers terminate in relation either to interstitial cells or spermatogenic elements.

Section of the sympathetic nerves to the testis results in degeneration of the seminiferous tubules.

27. *The development of the cross-striated myofibril in the heart muscle of the chick embryo.* MARGARET REED LEWIS, Carnegie Institution, Johns Hopkins Medical School.

By fixing the total chick embryo so that the heart is slightly extended, cross-striated myofibrils, of the same pattern as those found in embryos of two to four days, can be demonstrated to be present in the heart muscle of embryos of ten myotomes. This pattern is composed of two light bands and one dark band, all about the same width, and a wider gray band, arranged as follows: Light (J-band), dark (Z-band), light (J-band), and gray (Q-band). These bands are usually arranged in fibrils whose width, thickness, and length vary. Only a few fibrils are found at the stage of ten myotomes, but in embryos of fifteen or more myotomes the fibrils are numerous. A fibril extends in the same focus past several nuclei. The number, length and character of the fibrils formed depend upon the solution used in fixation.

Living chick embryos mounted in Locke's solution contain no fibrils in the heart muscle. The cross striations can occasionally be found spread out in an extremely thin layer on the surface of the cell. They have little depth when focused; they can be observed only in that region of the cell surface which is in focus, and never extend past several nuclei. The mitochondria can be clearly observed especially in preparations stained with Janus green. They are not elongated nor are they arranged in a definite row. In no case did a mitochondrion extend beyond the limit of a cell.

28. *The centriole and centrosphere in degenerating fibroblasts of tissue cultures.* WARREN H. LEWIS, Johns Hopkins Medical School.

In fibroblasts of tissue cultures undergoing vacuolar degeneration a centrosphere develops around the centriole. The centriole in the normal fibroblast lies close to the nucleus, the mitochondria do not appear to have any definite relation to it. As degeneration progresses, vacuoles increase in number and surround the enlarging centrosphere, and the mitochondria become radially arranged about it. The centrosphere may attain a size greater than the nucleus. There is much variation in the structure of the centrosphere in fixed specimens. The centriole may be surrounded by a finely granular zone varying in thickness in different cells. A homogeneous clear zone may intervene between centriole and granular zone or a homogeneous medullary and a homogeneous cortical zone may intervene. The finely granular zone may not be present. There are various other types of centrospheres and various gradations between them even in the same culture among cells side by side. Such differences indicate perhaps that the cells when fixed are often in different phases of metabolic activity. The coagulated cytoplasm is more dense about the centrosphere than about the nucleus, and the framework between the vacuoles radiates from it, not from the nucleus. This orientation of the various structures in the degenerating fibroblasts about the centriole and centrosphere, and their central location suggest that the centriole and not the nucleus is the center of metabolic activity or that there is an increase of the activity of the centriole in degeneration.

29. *Studies on the longitudinal muscle of the human colon, with special reference to the taeniae.* P. E. LINEBACK, Emory University.

This investigation covered a series of embryos ranging from 26 mm. to the new-born, in all, thirty-five different sizes were used. It also included experiments on the guinea-pig's caecum, and a few observations were made on the human colon in life.

The longitudinal muscle originated in the caudal end of the colon at about the 40-mm. stage and rapidly extends to the caecal end, first along the mesenteric line. This growth is closely followed by a complete layer which encases the tube, the whole being finished before definite taeniae appear. The mesenteric portion becomes thickened and develops into the first taenia, the other two subsequently appear as thickenings in the muscle wall and by the 105 mm. stage the three are definitely formed.

The production of taeniae of the adult state is based upon two factors and stages—a growth factor in an early stage and a functional factor in a later stage. The functional phase involved the combined action of the longitudinal and circular muscles and results in the production of sacculations as well as accentuating the taeniae. Further work is being done along this line.

30. *Brain repair in the rat vitally stained with trypan-blue.* CHARLES CLIFFORD MACKLIN and MADGE THURLOW MACKLIN, University of Pittsburgh and Johns Hopkins Medical School.

Healing aseptic wounds in the brains of rats, made by stabbing with a red-hot needle under ether anesthesia, were studied in a series of animals, covering the first seventy-four days of repair. Trypan-blue, in 1 per cent aqueous solution, was administered intraperitoneally two days before death.

Mononuclear phagocytes soon appear. They are quite numerous and contain much fat, blood pigment, etc., but very little dyestuff in comparison with the macrophages of injured muscle and connective tissue, such as appear in the vicinity of a fractured bone (Macklin, *Anat. Rec.*, Jan., 1918). Dye was found in them only during the first four days, and in this respect also they differ from the macrophages of inflammation of other tissues. Often the small vessels are outlined by rows of dye-containing cells and the use of the proper stains, and examination of fresh material shows that these cells are filled with fat. So close is the association between the amoeboid fat-containing phagocytes and the blood-vessels (the walls of which contain fat droplets) that the inference is strong that fat is thus gathered up and passed into the blood-vessels, its absorption being so accomplished. The trypanophilic cells are apparently developed largely from local neuroglia cells. Mitoses are abundant at the second and third days.

Other interesting features are the staining of the arachnoid cells of the lesion area, the homogeneous blue staining of the neighboring blood-vessels, and the pale diffuse staining of the area of inflammatory oedema during the early stages.

31. *The value of study of the gill-arch system in topographical anatomy.*

MATTHEW MARSHALL (introduced by Robert Retzer), University of Pittsburgh.

A study of the gill-arch derivatives simplifies the understanding of the relationships which obtain in the adult derivatives of these structures. A general statement of these simple relationships follows:

1. Interrelationships of derivatives of the same gill arch: A. The nerves are external to the artery and bar derivatives. B. The arteries are external to the bar derivatives. C. The muscle derivatives have inconstant relationships with the bar, artery, and nerve derivatives.

2. Interrelationships of derivatives of one gill arch with those of another gill arch: A. Derivatives of one gill arch when related to those of a higher gill arch are internal to them.

3. Relationships of the longitudinal aortic derivatives: A. Dorsal aortic derivative—internal to other gill arch derivatives. B. Ventral aortic derivative—external to other gill arch derivatives.

4. Exceptions can be accounted for on the basis of mechanics which comparative morphology indicates have been active.

32. *Note on the lung of the rabbit and of the guinea-pig.* WILLIAM SNOW MILLER, University of Wisconsin.

In a paper on the vascular supply of the pleura pulmonalis, published in 1907, I made the following statement: "the histological structure of a given organ may differ in animals of different species. . . . Because a given structure, or relation of structures, is found in, for example, rabbits, it is no criterion that the same structure, or relation of structures, will be found in rats, and the inverse is true." Recent studies have shown that, while in some animals the masses of lymphoid tissue present in the lung receive their blood supply from the bronchial artery, in the rabbit branches from the pulmonary artery are distributed to these masses, especially to those situated at the point where bronchi divide. This has an important bearing on any experimental work which concerns the vascular supply of these masses of lymphoid tissue.

Klein, many years ago, called attention to the presence of smooth muscle in the pleura of the guinea-pig. I have found that in the walls of the bronchial tree and in the walls of the blood-vessels there is, in this animal, proportionately a greater amount of smooth muscle than in the same structures in the animals ordinarily used in the laboratory.

33. *On the antiquity of certain histological elements of bone.* ROY L. MOODIE, College of Medicine, University of Illinois.

The first bone to develop on the bodies of vertebrates arose in the ectodermal tissues surrounding and capping the dorsal-fin spines of the Ordovician and Silurian vertebrates, the primitive sharks of the Paleozoic. Histological study of this ancient tissue reveals no evidence of Haversian systems or lamellae, but the lacunae with short, irregular canaliculi are to be found arranged in an indefinite way in the trabeculae surrounding the large vascular spaces.

The oldest known Haversian system occurs in a Devonian lung fish. Here the Haversian canal is large, the lamellae are definitely concentric, and the lacunae, with short canaliculi, surround the canal. There is no apparent communication between the canaliculi of the lacunae and the system has a primitive appearance.

The perforating fibers of Sharpey are seen for the first time in the history of the vertebrates in the mosasaurs of the Cretaceous, where they are arranged in large bundles and have considerable length. So far as ascertained they do not perforate the lamellae, and are apparently more numerous near the surface of the diaphysis. They are especially abundant in a surface lesion of osteoperisotitis and have not been seen in normal bone.

Osteoid tissue is seen in the same lesions, similar in all respects to the osteoid tissue of modern times. Osteosclerosis and osteohypertrophy occur sharply marked in the oldest known fractured bone from the Permian of Texas.

The indications so far observed in the material examined, covering nearly the whole range of the evolution of the vertebrates seen in geological history, show that the degree of development has been small

since the middle of the Paleozoic, the modern plan of bony tissues having been laid down very early in the history of the vertebrates.

34. *Amitotic karyokinesis and general histogenesis.* I. P. MUNSON, Ellensburg, Washington.

Mesodermal tissues are structurally related. Their obvious differences are due to the nature of the intercellular substances and the intracellular matrix. The cells of these tissues are distinct physiological centers, but they are not morphologically separated. Although, as aggregates, they seem to form solid masses, as in muscle, cartilage, and bone, they are made up of net-like membranes, either living cavities or superimposed. The anisotropic, structural elements of these membranes, consist of nucleus, centrosome, and sphere, with astral rays. These rays are continuous with the cytotreticulum which may be embedded in a solid, hyaline, or liquid matrix. The rays of one cell area are continuous with the rays of the neighboring cell areas. All the cells are connected by intercellular bridges of astral rays. Cell membranes do not exist. The boundaries are sometimes perceptible as enlargements of peripheral fibrils, or as granules deposited between the fibers at the original cell boundary. It might be designated as a syncytium, if the connotation of that term be modified accordingly. . . Endothelium, connective tissue, cartilage, bone, and muscle are organically connected with each other, as are the cells of these tissues.

Although karyokinesis as well as amitosis are rarely seen in these tissues when fully differentiated, cell multiplication is not as unusual as is generally supposed. By slow growth, the cells of these tissues divide in a manner that is neither karyokinesis nor amitosis, but rather a combination of those modes of cell division. A permanent and constant centrosome, which is a secreting organ as well as the center of cytoplasmic growth and the morphological center of the astral system, is found either at one pole of the nucleus, in the notch of the nucleus, or wholly within it. The nucleus divides by a process of cleavage, without forming a spindle. The centrosome may or may not divide at the same time. After the division of the nucleus, the centrosome usually moves in between the two halves of the nucleus and may then gradually divide. The two centrosomes gradually grow apart, but their astral rays continue to unite them after an equatorial plate of granules have been deposited between the two halves of the cell. In this way cell multiplication can go on without severing the connection between the cells. To avoid a new term, I have called this mode of cell division amitotic karyokinesis. In striated muscle the main facts are similar, though the details vary. A sarcomere bounded by the membranes of Krause is originally a cell with nucleus and centrosome.

Permanent and constant centrosomes also exist in some nerve cells. The neurofibrillae are extended astral rays. There is probably a similar continuity between neurons at the synapse, which marks the original boundary between the neurons. The tentative hypothesis is suggested that the neuraxon is a bundle of astral rays connecting nerve

cells, as similar fibers connect bone cells, and are responsible for the canaliculi of bone.

One conclusion to be drawn is that cells are organized, and that the cell theory is at fault when it fails to recognize the morphological continuity of protoplasm in many celled organisms. A mechanistic explanation of this cell organization is a problem suggested by the studies here announced.

35. Studies on the mammary gland. V. The effects of inanition on the developing mammary glands in male and female albino rats from birth to ten weeks of age. J. A. MYERS, University of Minnesota.

Severe inanition retards the growth of the milk-ducts of the female rat during the first week, but apparently does not completely stop their growth. In animals held at birth weight for a longer time the ducts cease to grow and remain in a condition slightly more developed than at the time of birth. If after the first week the gross body weight of the animal is allowed to increase so as to correspond with that of a normal animal of one week the milk-ducts fail to develop to the same extent as those of a normal animal of corresponding body weight. This also holds true if the body weight of the underfed rat is allowed to equal that of a normal animal of two weeks.

The growth of the milk-ducts of male rats is retarded by inanition in a manner similar to that of the female.

The nipple grows very little during inanition, being elevated above the surface only slightly in young rats starved severely for eight to ten weeks.

Severe inanition for a short time at an early age thus temporarily stunts the mammary glands. When the animal is refed the glands respond slowly. When the body weight during refeeding reaches that of a normal at the age of puberty, the milk-ducts are far behind those of the normal rat at corresponding body weight. That this stunting is not permanent is shown by the fact that the ducts ultimately attain the same stage of development as those of a normal animal, but at a much later period.

36. Studies on the mammary gland. VII. The distribution of the subcutaneous fat and its relation to the developing mammary glands in male and female albino rats from birth to ten weeks of age. JAY A. MYERS and FRANK J. MYERS, University of Minnesota.

In one series of rats the epidermis and corium were removed, care being taken to leave all the subcutaneous fat on the body. The carcasses were then placed in sudan III, scarlet red, or 1 per cent osmic acid. The subcutaneous fat became beautifully differentiated from the surrounding tissues. In another series of rats care was taken to remove all the subcutaneous fat with the skins which were placed in one of the above-mentioned stains and later cleared in glycerin.

The subcutaneous fat is not deposited uniformly over the body, but tends to accumulate in pads in the regions which the ducts of the mam-

mary glands already or will later occupy. In the inguinal region there appears on each side a fairly definite pad of fat which is thickest cephalad to the ilium. This has been designated the inguinal fat pad. It extends medially then caudad over the pubis and approaches the anal region. The milk-ducts of the abdominal and inguinal mammary glands ramify in the inguinal fat pad. In the thoracic region a mass of subcutaneous fat is deposited on each side of the body. This thoracic fat pad extends cephalocaudad from the region of the axilla nearly to the costal margin. From the dorsal midline it extends ventrad, but ends a considerable distance from the ventral midline. The cervical fat pad extends over the root of the neck and is connected with the thoracic pad. The milk-ducts of the three thoracic glands ramify in the thoracic and cervical pads of fat.

37. *A comparison of the functional adaptation of the middle-ear region in birds and mammals to barometric fluctuations.* A. G. POHLMAN, St. Louis University.

It is well known that displacements of the drum membrane in mammals do not give rise to correspondingly great movements at the Fenestra vestibule, and the simple columellar apparatus in birds through a system of intrinsic bendings accomplishes the same function. It is difficult to believe either system has a transforming function in relation to sound waves. The drum, in mammals, tends to displace lateralward when muscular tension is relaxed, while in birds muscular relaxation is accompanied by medial drum displacement. A positive pressure in the external auditory canal is resisted only by drum elasticity, while the weight of the medially displacing ossicular chain is held up in part by the M. stapedius. The negative pressure in the external canal is actively resisted by the M. tensor tympani and, probably because of the character of the joint surface between Malleus and Incus, is not accompanied by marked excursion of the Stapes. In birds, positive pressures in the external canal are resisted by the M. tensor tympani which at the same time limits medial displacement of the columellar apparatus against the Fenestra vestibule, while negative pressure is passively resisted through elastic ligaments, columellar form, and the possibility of a shearing off the external auditory canal. Both forms display excellent adaptation to fluctuations in barometric pressure without materially influencing the pressure in the perilymph. The Membrana tympani secundaria may be considered a compensation opening to allow for mass displacements in the perilymph as a result of movement in the Stapes or columella—a function which it seems to exercise in the Anurans, although located entirely outside of the tympanic cavity.

38. *The mitochondrial content of the principal cells of the central nervous system during hibernation in the woodchuck (Marmota monas).* A. T. RASMUSSEN, University of Minnesota.

Since profound hibernation is attended by such a marked reduction in the activities of many organs, including the nervous system, this

dormant condition naturally suggests itself as being of value in connection with attempts to unravel the tangled question of the functional significance of mitochondria.

Having found no record of such investigations on the nervous system during lethargy, it is interesting to note that, as far as results have been obtained in research now in progress on one of the species of American marmots, the mitochondria in the chief cells of the brain and spinal cord show no noticeable variation in number, size, shape, or grouping during hibernation. The following cells have been examined: somatic motor cells from the ventral horn of the spinal cord, visceromotor cells from the lateral horn of the spinal cord, cells of nucleus gracilis and of nucleus cuneatus, Purkinje cells in the vermis of the cerebellum, cells from the nucleus of the superior colliculus, Betz cells from the motor cortex and mitral cells of the olfactory bulb.

The conclusions are based upon a series of fifteen adult animals, five of which were sacrificed before hibernation commenced, five toward the end of the dormant period, and five at various intervals after waking up and becoming active.

The work is being extended to other nerve cells and to the entire glandular system.

39. *Experimental inhibition of neural concrescence and some conditions resulting.* FRANKLIN P. REAGAN, Princeton University and the University of Illinois Medical School.

Careful desiccation of the vitelline membrane of the chick embryo serves to stiffen that structure. If this desiccation be carried out at a time prior to neural concrescence, the neural tissue adheres to the membrane, rendered powerless to form folds. Usually the brain tissue back to the midbrain becomes tubular. Caudad to this as far as the pelvic region the neural tissue often remains flat, flanked on either side by feather-germs after those structures have appeared in development. The roots of the spinal nerves course dorsoventrally. What would ordinarily be vertebral column remains as flat layer of skeletal tissue, ventral to the neural tissue and laterally coextensive therewith. Dorsal commissures are of course eliminated. Oedematous condition ventral to the neural tissue is generally attended by unusually numerous lymphatics. In many cases the anterior brain tissue is thrown into many folds and pouches which may be strikingly symmetrical. In the diencephalic folds diminutive retinæ have been located, far removed from body-wall ectoderm which has failed to produce lenses. Such embryos can be reared to stages where interesting results by direct stimulation might well be obtained.

40. *Muscle and fascia.* ROBERT RETZER, University of Pittsburgh.

A muscle whose fibers are arranged parallel to its long axis is weaker than a muscle of the same volume whose fibers are arranged at an angle to its long axis. The former contains longer fibers which permit of a greater degree of contraction, and it is found that a muscle of this

group always is inserted where it may have advantageous leverage to compensate for its weakness. Reverse holds for group whose fibers are arranged at an angle. The greater the angle the less is extent of contraction and with volume as a constant the stronger the muscle. It is inserted where leverage is disadvantageous.

Coarseness or fineness of muscle fasciculi have nothing to do with comparative strength of muscle. A muscle that has coarse fibers invariably has more than one function. The coarser the fibers the more widely divergent are these functions. With these muscles there is no action possible that would allow all the constituent fasciculi to contract simultaneously and to the same degree. Whenever contraction and relaxation in a muscle occur at the same time and to the same degree, the muscles are fine fibered.

The coarseness or fineness of fasciculi is dependent upon the amount of fascia interposed between the fasciculi. Fascia is developed to prevent friction between the muscle fasciculi. Muscles are divided into morphological entities not by the nerves which innervate them, but by the function they perform. The more divergent these functions are, the more fascia is interposed between the muscles.

41. Laws governing the pathways of peripheral nerves. ROBERT RETZER, University of Pittsburgh.

A physiologic explanation that is purely theoretical and without experimental evidence is suggested to explain the following laws which are found to be true for the peripheral nervous system:

Law 1. Any nerve innervating a given muscle is in part of its course in contiguity with the nerves that supply all the other muscles necessary for the proper performance of the function of that muscle.

Law 2. In automatic coördinate movements, all muscles which must act first in a given movement are innervated first, i.e., they have shorter nerves.

Law 3. All nerves whose activity is necessary for the coördinate performance of a given movement are enclosed, for a part of their course, in a sheath, and the longer the ensheathment the more constant is the coördination of these nerves.

Law 4. All peripheral nerves are contiguous in part of their course with at least another peripheral nerve. The greater the number of nerves with which a given nerve is in contiguity the greater is the complexity or extent of the coördination of the areas supplied by these nerves.

Law 5. Every motor nerve is ensheathed, for a part of its course, with a longer sensory nerve.

Law 6. The same trunks of nerves, whose branches supply the groups of muscles moving a joint, furnish also a distribution of nerves to the skin over the insertions of the same muscles, and the interior of the joint receives its nerves from the same source. (Hilton.)

42. *Some peculiarities of the growth of the human uterus.* RICHARD E. SCAMMON, University of Minnesota.

A study of a considerable series of measurements of the length of the uterus shows that the organ passes through several phases of growth which are not observed in the other organs of the body. During fetal life the growth of the uterus is not markedly different from that of most of the other fetal organs, but immediately after birth it loses nearly one-third of its natal length. This loss in length, which has been previously described by other authors, is completed before the end of the first trimester. From this time until about six years there is little change in length. At about six years there is a short period of growth during which the organ increases about 20 per cent in length. This growth period is followed by a second period of quiescence which continues, on the average, until about the eleventh year. The pubertal growth period is very variable, but it is usually both earlier and shorter than is generally assumed. Statistical studies indicate that, in the majority of cases at least, most if not all of the so-called pubertal growth takes place before the menarchy. An examination of the available data on the weight of the uterus indicates that the changes of the organ in weight are much the same as the changes in length except that there is some increase in the weight of the organ throughout the latter part of childhood. The relative weight of the uterus is quite variable, but it evidently decreases throughout early postnatal life. (Lantern.)

43. *Developmental rate and the perfection of structure.* CHARLES R. STOCKARD, Cornell University Medical School.

Variation in the direction of increased developmental rate is more limited and more difficult to experimentally bring about than is variation toward a decreased or slower rate.

The acceleration of developmental rate that may be induced in early embryos tends to increase the perfection of the resulting individual. The ideal rate of development is probably somewhat faster than the average generally followed.

The limits of retardation in the rate of development are extremely wide. Development may be slowed down to almost zero or apparently stopped for long or short periods of time without noticeably injuring the embryos of many species. However, when the rate of development is retarded, but not entirely stopped, at certain critical periods and development is allowed to proceed at the diminished rate for some time, most serious structural anomalies are induced.

Double monsters very likely result from a slowing down of the rate at a time when the primary embryonic bud should arise. Normally, the initial appearance of the primary embryonic bud probably suppresses other buds which potentially exist. The distance apart of two buds on the blastodisc determines the degree of doubleness of the resulting individual. Unless the two buds arise almost simultaneously, they are unequal in their later developmental rates and structural conditions. When one bud obtains the start, this start constitutes a

supremacy which almost invariably allows the leading bud to develop into a perfectly normal specimen, and invariably defeats the possibility of normal development on the part of the slower bud.

44. *A very young monozygotic twin.* GEORGE L. STREETER, Department of Embryology, Carnegie Institution.

The specimen reported consists of an amniotic vesicle 0.1 mm. in its largest diameter, with a detached yolk-vesicle 0.03 mm. in diameter. The amniotic vesicle is spherical in form and possesses an ectodermic wall the embryonic and amniotic portions of which can be clearly distinguished. The two vesicles are enmeshed in the loose mesenchyme in the region of the body-stalk of the co-twin. The latter is a well-preserved, normal embryo in the primitive-groove stage, having an embryonic plate 0.92 mm. long, 0.78 mm. wide. The greatest external diameter of the chorion enclosing the two embryos is 9 mm.

The relation of the smaller twin to the larger is of the character that would be expected when the primary embryonic mass within the chorionic ectoderm had undergone division into the primordia of the two embryos, and the specimen thus tends to substantiate that theory of the origin of such twins.

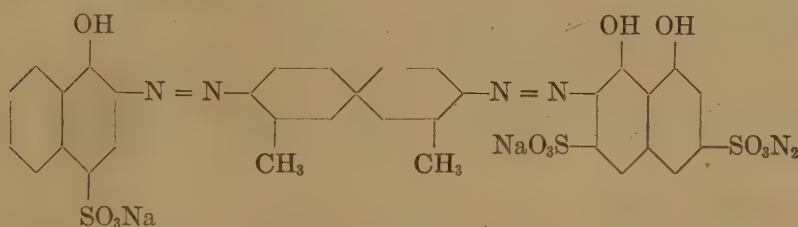
45. *On the behavior of the mammary epithelial cell towards vital dyes in various functional epochs of its life cycle.* MONROE SUTTER, University of California.

Various researches in this laboratory have in the last few years been directed towards the solution of the phenomena of vital staining with dyes of the acid azo class. These substances can be said to demonstrate in a spectacular way the power of specific cells and tissues to ingest, store, and concentrate material which is in an analogous physical and chemical state with such dye solutions. This conclusion would appear to mark an advance in our understanding of the 'vital staining' reaction, for theories of the staining of living protoplasm by virtue of unknown specific chemical or physical combinations have hitherto dominated the field. The vital azo dyes demonstrate the capacity of cells to be penetrated and to segregate within their protoplasm, accumulations of such substances.

In particular, the differing reaction of various closely related cells to these tests has received attention in this laboratory. There has also been undertaken a comparative study of the vital dye reaction displayed under various experimental conditions by the same cell type. This histophysiological agent has thus given us proof of very significant changes in the behavior of the same cell type when submitted to various experimental conditions. In some instances we are able to clearly relate these to impaired vitality of the cells.

The following communication deals with the mammary gland in the rat. Certain of the vital acid dyes are always deposited in some few of the epithelial tissues, though such phenomena constitute exceptions to the rather rigid rule of the exclusion of the epithelia from

participation in the 'vital staining' effect. The hepatic parenchyma, adrenal cortical glomerulosa, glandular pituitary, renal tubular, ovarian luteal, and the mammary epithelia are to be mentioned here. Many dyes of the acid azo series are deposited to some extent in the mammary epithelial cell. On the administration of some of these dyes, however, the actively functioning mammary epithelium refuses to store the dye-stuff, even though it does so when the gland is in a resting condition. In such cases, impairment and regression of the gland produced by cessation of suckling promptly leads to a different behavior on the part of these cells. They now accumulate large vital dye 'deposits.' This is the case with the blue dye produced from the diazotization of ortho toluidine and its linkage with a molecule each of chromotrope and the Neville-Winther acids.¹



Unlike the above dye, trypan-blue produces cellular deposits in the resting, the developing, and the actively functioning mammary epithelial cells, but the number of the dye 'granules' is greatly increased during regression. Wieszeniewski has secured a similar reaction on the part of slightly injured renal epithelium after clamping momentarily the renal artery.

The production of cytoplasmic dye deposits in every mammary epithelial cell in this way has demonstrated in an irrefutable manner that each cell maintains its integrity throughout the lactation period, even though the peripheral part of the cytoplasm is discharged en masse in milk elaboration and secretion.

46. *On the experimental production of edema by nephrectomy.*² W. W. SWINGLE (introduced by C. F. W. McClure), Princeton University.

These experiments were performed in order to test the view recently advanced by C. F. W. McClure (*Jour. Gen. Physiol.*, vol. 1, no. 3) that edema may be due to block in kidney function of some sort.

1. The glandular portion of both pronephroi was extirpated from 6-mm. larvae of *Rana sylvatica*, at about the time when this organ first

¹ This dye has been employed by Evans and Long to 'mark' corpora lutea of known age in order to test their survival under various circumstances, and by Corner and Hunri (*Amer. Jour. Physiol.*, vol. 46, 1918) for similar purposes.

² Essentially similar observations have been made by Ruth B. Howland on the embryos of *Ambystoma* (*Proc. Nat. Acad. Sci.*, vol. 2, 1916) which the above results confirm. I regret that I was unaware of the existence of this paper when my abstract was sent to the press.

becomes functional. Twenty-four to thirty hours later the larvae were all edematous—in fact, swollen to such an extent that rupture of the body wall occurred in many.

2. The glandular portion of the pronephros (on one side only) was extirpated in a second set of larvae. Slight edema developed within twenty-four hours, confined chiefly to the side lacking the kidney. The remaining kidney underwent great hypertrophy. The larvae all survived. Four days later the edema disappeared, due to the functional development of the mesonephros.

3. The Wolffian ducts of one other set of larvae were severed in the posterior part of body and portions cut out. Edema developed within twenty-four hours with great distention of the lymph sinuses and body cavity.

4. In another set of 6-mm. larvae, the Wolffian ducts were severed cephalad of the cloacal opening, the tubes dissected anteriorly, and hung outside the larva through the incision in the body wall. The glandular portion of the kidney was left intact. Those larvae which survived the operation for thirty-six hours showed practically no edema. The kidneys functioned normally despite the abnormal position of the ducts.

47. *The relation of the cartilaginous otic capsule to the facial nerve.* R. J. TERRY, Washington University School of Medicine.

The bony skeleton of the ear of mammals is tunneled more or less extensively by the facial nerve in its exit from the cranium. The cartilaginous otic capsule also of mammalian embryos is traversed by the facial nerve. Studies of the development of the otic capsule of mammals have brought forth evidence of at least two different elements entering into its composition: one, the cartilaginous wall enclosing the epithelial cochlear canal and semicircular ducts, the other, the supra-facial commissure, probably a parietal derivative. Between these elements is the canal for the facial nerve, in such position that the proper otic capsule is posterior, the suprafacial commissure anterior. In the embryos of reptiles and amphibians the facial nerve passes cephalad of the otic capsule in its exit from the chondrocranium through an opening between the otic capsule and some part of the floor or lateral wall of the skull, that is, a parietal part. This, which is regarded as the primitive relation of the facial nerve to the otic capsule, is masked in adult amphibians, reptiles, and mammals first by fusion of the parietal element with the otic capsule and later by ossification.

48. *The structure of the clasmatocyte.* HARRY W. VANCE (introduced by W. H. Lewis), Johns Hopkins Medical School.

Clasmatocytes are abundant in cultures of subcutaneous tissue, and in fixed specimens some of them are flattened out on the under surface of the coverslips in such a manner as to enable careful studies to be made of their structure. The most striking features are the large centrosphere, the large vacuoles, and the eccentrically placed nucleus.

The large finely granular centrosphere occupies, in most of the flattened cells, approximately the center of the cell. At the center of this centrosphere is often seen a single or double centriole. The centriole when visible is always separated from the nucleus by a distance equal to or greater than the radius of the centrosphere, and this distance may be greater than the short or even long diameter of the nucleus. The fine granules of the centrosphere in the specimens so far studied come close up to the centriole, when the latter is visible. The periphery of the centrosphere is continued into the cytoplasmic framework, lying between the vacuoles which otherwise fill up the peripheral regions of the cell. There are often indications of radiations in the centrosphere which seem to continue into the cytoplasmic framework. The nucleus is almost always crowded off to one side of the cell, often touching the edge. In many cases vacuoles separate the centrosphere from the nucleus. The centrosphere with its centriole thus seems to be the center of activity, the dynamic center of the cell according to Boveri.

49. *The effect of intravenous injections of various concentration upon the central nervous system.* LEWIS H. WEED, Capt., M.C., and PAUL S. MCKIBBEN, 1st Lt., San. C., Army Neuro-Surgical Laboratory, Johns Hopkins Medical School.

The intravenous injection of solutions of various concentrations have been found to alter markedly the pressure of the cerebrospinal fluid. Strongly hypertonic solutions of the common sodium salts (chloride, sulphate, bicarbonate) or of glucose, on intravenous administration, give an initial rise in the pressure of the cerebrospinal fluid followed by a profound and enduring fall, frequently to below zero. Similar injections of hypotonic solutions (distilled water) cause a marked and persisting rise in the pressure of the fluid. Ringer's solution, in control isotonic injections, alters the pressure of the fluid only during the period of introduction; the pressure quickly returns to its former level.

The brains of animals receiving injections of the hypotonic or hypertonic solutions are considerably changed in volume. The brain of the animal given an intravenous injection of Ringer's solution is not abnormal. After the intravenous injection of the concentrated salt solution, the brain becomes shrunken, the gyri more rounded, and the sulci widened. The intravenous injection of water causes marked swelling of the brain, flattening of convolutions, and obliteration of sulci. If these changes are brought about in a trephined skull, with opened dura, the brain of the animal receiving water protrudes in a marked herniation, while with the concentrated salt a tremendous shrinkage of the brain away from the skull is noted. With Ringer's solution, in such experiments, the brain maintains a gentle convexity in the trephine opening.

Such alterations in brain bulk are independent of vascular changes and persist after formalin fixation.

50. *Influence of the ovaries upon the production of artificial deciduomata; confirmatory studies.* GEORGE W. CORNER and STAFFORD L. WARREN, University of California.

One of the few recent clues toward solving the perplexities of interaction between uterus and ovaries was given in 1907 and 1908 by Leo Loeb, in his discovery that at certain stages of the reproductive cycle of the female guinea-pig, injury to the uterine mucosa leads to the formation of a tumor at the site of trauma which closely resembles in cellular structure the maternal portion of the placenta.

This reaction of the endometrium can be elicited only during a limited time, from two to nine days after ovulation; it does not occur in the absence of the ovaries, even if these be left in place until after ovulation has occurred. From these facts Loeb believes that the young corpus luteum formed at the time of ovulation develops a hormone which in some way sensitizes the uterine mucosa in time to receive the ovum and to participate in placenta formation. The artificial stimulus of his experiment merely imitates the trophic action of the early embryonic ectoblast.

Evidence confirming these statements has been brought forward by Robert Frank ('11), using rats. In view of the importance of the subject and the increasing use of this species in the laboratory, it seems worth while to add the results of further studies with rats.

Pregnant animals were selected from a colony of albino rats (*Mus norvegicus*) slightly admixed with brown strains. They were allowed to give birth, in order to fix the date of ovulation, which invariably occurs within twenty-four hours after parturition. Seven to eight days after parturition (therefore, six to seven days after ovulation), the abdomen was opened under ether anaesthesia and the uterus traumatized by the insertion of a small foreign body, such as a fine short piece of glass, into the lumen; by the passage of a silk suture through the uterine wall, or merely by scratching the mucosa with a needle inserted through the wall.

In seven successive animals treated in this way, and then killed four or five days after operation, the traumatized areas were found to present large soft solid enlargements of dark congested appearance, in greatest diameter more than twice as thick as the intervening parts of the uterus. In color and texture the tumors greatly resembled the enlargements of early pregnancy in the rat. Microscopic sections through them showed the uterine mucosa to have been replaced by a solid mass of cells varying from spindle-shaped to large oval outline. Many nuclei were in mitosis; an occasional cell was polynuclear. In some specimens small local areas of degeneration were seen. Even when the traumatizing object, suture thread or glass, had been left in place, the microscope showed that we were not dealing with the familiar foreign-body reaction of tissues in general, but with the production of true decidual cells.

Seven other rats were treated in exactly the same way, except that after waiting from twenty-four to forty-eight hours after parturition

(to permit ovulation) the ovaries were removed. On the seventh or eighth day the uteri were traumatized, and four or five days after the second operation the rats were killed. In none of these animals did any enlargement at the site of trauma take place.

In two animals the ovaries were merely separated from the uterus by cutting between ligatures placed near the tubal extremities of the uterine horns. In these experiments the placenta-like tumors developed, showing that the influence of the ovaries is exerted through the bloodstream or possibly the nervous system.

Recent determination of the ovulation cycle of the white rat by Long and Evans ('19) shows that this animal is not altogether suitable for testing the whole of Loeb's hypothesis, since oestrus and ovulation recur at periods of four to eleven days in different individuals (usually five days), while corpora lutea persist at least eight weeks before degeneration. Thus it would seem that the uterus must be almost constantly subject to the influence of the ovaries, and that an attempt to relate the phenomenon of deciduoma formation to the definite stages of the corpus luteum would be hopeless with this species. Our few experiments with time intervals differing from those given above as the optima gave varying results, as might have been expected.

It was hoped that it might be possible to elicit deciduomata in rats deprived of their ovaries immediately after ovulation, if early corpus luteum tissue from another species were provided. Recent observations of one of the present authors (Corner, '19) upon the development of the corpus luteum of the sow, permitted the collection of a plentiful amount of tissue from the first week after ovulation, but its administration to a small series of rats, by mouth in the fresh state, by abdominal injection after desiccation, or in alcoholic extract, has so far given negative results.

51. Sidelights from early abnormal conceptuses. A. W. MEYER, Stanford University.

An examination of some early human conceptuses suggests that the amnion is formed by cavitation as assumed, and that the chorionic and umbilical vesicles at least have some power of independent unassociated growth.

Early death of the embryo apparently sometimes is due to failure of proper rotation of the chorionic vesicle, in consequences of which the belly stalk becomes located opposite the reflexa. Hence the eccentric forms of insertion of the umbilical cord may in part at least be due to faulty orientation on part of the implanting conceptus.

The presence of undoubted isolated vascular rudiments in the villi of some young specimens also would seem to suggest that the development of villous vessels is not necessarily wholly centrifugal nor dependent entirely upon the vascularization of the embryo itself. This conclusion would seem to be unavoidable even if one could assume that normal and abnormal development follow fundamentally different plans.

Although the occurrence of pathologic, or at least of abnormal, unfertilized ova within the ovaries is not at all unlikely, the lack of directly confirmatory evidence emphasizes not only the need of further study of these organs, but especially the critical examination of both early abnormal and apparently normal conceptuses. Since early embryos usually are obtained from abortuses it would perhaps be safer to assume that many of these are not wholly normal in form and should be regarded with suspicion.

DEMONSTRATIONS

1. *Eosinophilic myelocytes and basophilic cells in the thymus of post-natal pigs.* J. A. BADERTSCHER, Indiana University.
2. *Series of hind limbs of pig embryos showing the genesis of bone, muscle, and joints.* EBEN J. CAREY, Creighton Medical College.
3. *Hands of polydactyl negro twins.* C. H. DANFORTH, Washington University School of Medicine.
Photographs and drawings showing points of similarity and difference in the anatomy of the hands from a pair of negro infants.
4. *A 3-mm. human embryo.* C. L. DAVIS, George Washington University Medical School.
5. *Sections of Crepidula plana showing different developmental stages of the gonad.* HARLEY N. GOULD (introduced by Dr. Robert Retzer), University of Pittsburgh.
6. *Sections of guinea-pig cochleae showing the normal and the lesions produced by detonations.* STACY R. GUILD, University of Michigan.
7. *A. Models of the digestive system, heart, and the jugular lymph sac of the 7-mm. opossum embryo. B. Dissections and stereophotographs of opossum embryos.* CHESTER H. HEUSER, The Wistar Institute of Anatomy.
For each embryo the final stage of the dissection was planned in advance, but for records and future study numerous sets of stereophotographs were made during the progress of the work. Details in the photographs are much better seen if enlargements be made in the form of transparencies. Also instructive views of topographic relations can be obtained by preparing 'ghost' pictures from two pairs of negatives. This requires double exposures and the registry must be accurate, or similar effects can be had by making the left positive from one set of negatives and the right from another.
8. *Thyroid and hypophysis transplants in Amphibia.* E. R. HOSKINS and M. M. HOSKINS, University of Pittsburgh.

9. *A human embryo with 2-3 somites.* Slides and drawings. N. WILLIAM INGALLS, Western Reserve University.

Embryo no. 1878 of the Carnegie Collection has a length of 1.38 mm. and a well-marked dorsal concavity. A short, 0.13 mm., primitive streak, traces of dorsal opening of archenteric canal, and small, plug-like cloacal membrane are present. Indications of first branchial cleft; well-defined, hollow thyroid. Open neural tube, early optic pits, otic plates, and ganglion crest of N. V. Three somites are indicated on left side, two on right; anterior and posterior limits uncertain. Long, distally dilated allantois, amniotic duct. Blood-vessel formation in body-stalk, yolk-sac, and embryo; vascular connections of these areas very attenuated if not wanting. Continuity of vitelline plexus and omphalomesenteric veins in embryo doubtful. Union of these latter veins to form plexiform, not yet entirely pervious heart, separating again soon to form open, endothelial lined, plexiform ventral aortae and first arches. Wide space between heart and myoepicardial mantle, bridged by numerous, fine, regularly dispersed protoplasmic fibrillae. Beginning differentiation in form of mantle, early stage of dorsal recesses of pericardial coelom. Dorsal aortae not completely formed, nor patent throughout, terminating caudally in single vitelline (umbilical) artery which forms (right side) a very slender connection with posterior part of vitelline plexus, left side uncertain. Vessels in body-stalk large and numerous, only small connection with chorionic vessels; connections with vitelline plexus indirect and ill-defined. Short left umbilical vein, entirely unconnected with other vascular anlagen, in somatopleure at junction with amnion. Throughout, various stages in vasculogenesis.

10. *a. Microscopic preparations of wing muscle of wasp, fly, mantis, and elater. b. Intercalated discs in human leg muscle.* H. E. JORDAN, University of Virginia.

11. *Models of the Herzog embryos.* FREDERIC T. LEWIS, Harvard Medical School.

12. *The centriole and centrosphere in degenerating fibroblasts.* WARREN H. LEWIS, Johns Hopkins Medical School.

13. *Vital staining of aseptic brain wounds.* C. C. MACKLIN, University of Pittsburgh.

14. *Wax reconstructions (Born) of the pronephros in oedematous frog larvae.* C. F. W. McCLURE, Princeton University.

15. *Model and drawings showing fate of gill-arch derivatives on the human body.* MATTHEW MARSHALL (introduced by Doctor Retzer), University of Pittsburgh.

16. *A. Slides: 1. Permanent centrosomes connected by intercellular bridges formed from astral rays. 2. Constant and permanent centrosomes in nerve cells. 3. The centrosome as a secreting organ. 4. Cells with centrosome dividing without formation of spindle by amitotic karyokinesis. B. Plates: Twenty plates with 250 figures, showing centrosomes in tissue and sex cells of all the important classes of animals. J. P. MUNSON, Ellensburg, Washington.*
17. *Monozygotic twin lambs. Craniopagus. B. D. MYERS, Indiana University School of Medicine.*
18. *The effects of inanition on the developing mammary glands in male and female albino rats from birth to ten weeks of age (cleared preparations and stained sections). J. A. MYERS, University of Minnesota.*
19. *The distribution of subcutaneous fat in male and female albino rats from birth to ten weeks of age (stained and cleared preparations). J. A. MYERS and FRANK J. MYERS, University of Minnesota.*
20. *A. Graphic charting of anomalies in pig series. a. Right subclavian artery from the aorta descendens and defect in the aortic-pulmonary valve. b. Persistent of symmetrical right and left pulmonary arteries to the lungs. B. Dissection to show the effect of positive and negative pressures in the external auditory canal upon the columella and elastic ligaments of the middle-ear region in Gallus. A. G. POHLMAN, St. Louis University.*
21. *Manuscript, drawings, and text of the late Professor Sheldon's book on the nervous system. ROBERT RETZER, University of Pittsburgh.*
22. *Cleared preparations and stained sections illustrating the changes in the mammary gland of the albino rat during the second half of pregnancy. F. L. ROBERTS (introduced by J. A. Myers), University of Minnesota.*
23. *Graphs illustrating the growth of the human stomach. RICHARD E. SCAMMON, University of Minnesota.*

A series of graphs illustrating the changes in the absolute and relative weight, the area of the mucous membrane, and the cubic capacity of the human stomach from early life to maturity.
24. *A simple mounting for demonstration slides. RICHARD E. SCAMMON, University of Minnesota.*

A simple mounting card for special or demonstration slides for class use.
25. *Graphs illustrating the weight and length of the new-born child in Europe. RICHARD E. SCAMMON and STANLEY H. HAYNES, University of Minnesota.*

A series of graphs and maps illustrating the average weight and length of the new-born child in the various districts in Europe. Graphs

showing the variation in weight and lengths in these same districts. Tables of comparison of the weight and length of new-born European children with the weight and length of new-born Caucasian children in other countries and new-born children of other races.

26. *A. Reconstructions of the axial artery of the lower extremity of a 6-mm. human embryo. B. A collection of photographs of anomalies of the arteries of the human lower extremity.* H. D. SENIOR, New York University Medical College.

27. *Anatomical drawings.* R. M. STRONG, Loyola University School of Medicine.

28. *Museum jars of terra-cotta.* R. J. TERRY, Washington University School of Medicine.

These jars are adapted to specimens which present one surface of interest and which require only slight illumination from above and at the sides. Two forms have been made: 1) a discus for the exhibition of frozen sections and similar large flat specimens; 2) rectangular jars for the usual objects in anatomical collections. The glass front is secured either by clamps or by cementing, depending upon the size of the jar. The terra-cotta is tinted and by glazing made impervious to fluids. These jars are not expensive, and since they can be made at any pottery are easily obtainable. The fact that light does not enter from the back, as in the case of the glass jar, and strike the eyes of the observer is a distinct advantage.

29. *Early human ovum, specimen and drawings.* F. W. THYNG, New York University Medical College

30. *The structures of the clasmatocyte.* HARRY W. VANCE (introduced by W. H. Léwis), Johns Hopkins Medical School.

31. *A simple method of injecting blood-vessels so as to reveal them in Roentgenograms. Demonstration of stereoscopic Roentgenograms of the vascular supply of some of the joints.* C. R. BARDEEN, University of Wisconsin.

To the alcohol, carbolic acid, glycerine mixture now in common use for preserving cadavers, barium sulphate may be added and the mixture injected in the usual way. If the mixture with barium sulphate is not too thick, the embalming fluid will flow readily throughout the cadaver and diffuse so as to preserve the tissues indefinitely. The barium sulphate remains in the blood-vessels and makes these opaque to the Roentgen rays. If the cadaver is subsequently dissected, the larger vessels should be tied off before cutting them, since the mixture in them remains soft. The barium-sulphate mixture in the smaller vessels causes little difficulty during subsequent dissection.

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Resumido por el autor, Frank Blair Hanson.

Demostración de las capas germinales.

El autor propone los estados denominados A-H por Balfour en el desarrollo del embrión de tiburón como el mejor material fácil de obtener para demostrar a los estudiantes de cursos elementales el origen de las capas germinales. Los embriones correspondientes a dichos estados se cortan transversalmente, seleccionando cierto número de cortes practicados a diferentes niveles del cuerpo del embrión. Las capas germinales están bien separadas unas de otras en la cavidad de segmentación y, por consiguiente, se evitan las dificultades o confusiones con que tropieza el estudiante cuando intenta analizar las capas germinales de los anfibios.

Translation by José F. Nonidez
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ON TEACHING THE GERM LAYERS

FRANK BLAIR HANSON

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FIVE FIGURES

The course in Comparative Embryology for premedical students as usually organized around the chick and the pig seems to lack on the laboratory side material for certain fundamental conceptions of the earliest development, without which the student finds himself at sea when attempting to study later stages, such as the 33-, 48-, and 72-hour chick and the 10-mm. pig. That this lack is felt is evidenced by the introduction at the beginning of many courses of the frog's egg or the egg of some teleostean form for the study of the cleavage stages and the rise of the germ layers. That these are sufficient for the cleavage stages is undoubted, but when the beginning student attempts to unravel the germ layers of the amphibian embryo and keep track of the history of the various cavities therein (segmentation, archenteron, and coelome), his conclusion is apt to be that the germ-layer theory is more theory than fact.

The writer wishes to call attention to Balfour's stages A to H of the shark embryo as probably the best material available for teaching the germ layers. In our Comparative Embryology class at Washington University the cleavage stages are taught from *Amphioxus* slides, also the blastula and gastrula. Then the shark, Balfour's stages, are taken up in toto mounts and transverse sections. The transverse sections are made up into sets, each set containing a slide from each stage through the same region of the body. For instance, one set (figs. 1 to 5) is through the middle of the body; another set gives the stages of the brain; a third near the anterior end of the foregut, and the last through the tail. Four sets are used at these four levels, others could be added or substituted.

In the set herein illustrated there is never any confusion in the student's mind as to the identity of the germ layers, for there is no confusion of the germ layers themselves. They are perfectly separate and distinct, lie at considerable distance from each other, and the first three cavities of the embryo are



always definitely demarkated and followed from stage to stage without difficulty.

The origin of the mesoderm from the entoderm of the gut is conclusively shown, for this form at least. The schizocoele is formed before the student's eyes, and a few words of explanation contrasts this with an enterocoele. The development of the spinal cord in its earliest stages, the infolding of the neural plate,

its origin from the ectoderm, are all told simply, quickly, and clearly in the set of five slides indicated.

The notochord is seen to arise out of the dorsal midline of the entoderm of the digestive tract, and its subsequent history is all contained within this same set of slides.

The shark has been used here for several years and with uniformly good results. A diagrammatic series of figures could hardly be devised that would more clearly show the steps of these early and important stages, while these have the advantage of being the sections of an actual animal. From this an easy transition is made to the chick and later the pig.

It has been the writer's experience that, judging from the final examination papers, this part of the work relating to the rise of the germ layers based upon shark material has left one of the most vivid impressions of the entire course. It has been likewise his experience that when only amphibian material was used, this remained the muddiest part of the course. By the use of specific colors for each germ layer, the differentiation and contrast is heightened, and the student soon comes to associate automatically each germ layer and its respective color.

Balfour's stages of the Elasmobranch embryo may be secured from several dealers in such supplies, and are not expensive if one cuts his own sections. To install sets of the stages outlined above costs approximately one dollar per student. Since the same slides are used year after year, in subsequent years there is no expense, the student paying for all breakage.

Figures 1 to 5 are camera-lucida drawings of the set of slides through the midsomites. It has been found advantageous to have the student study successively sections from all the stages at the same level of the body, rather than study the different levels in each stage before passing to the next.

Resumido por el autor, Frank Blair Hanson.

El coracoides de *Sus scrofa*.

El proceso coracoideo falta en el cerdo. Existe una porción subcoracoidea que participa en la constitución de la fosa glenoidea y es homóloga del subcoracoides del hombre. El subcoracoides se ha considerado como una simple epífisis, pero puede muy bien corresponder a la epífisis del metacoracoideo, como ha indicado Gregory. En la escápula del cerdo hay solamente un centro de osificación en la parte coracoidea.

Translation by José F. Nonidez
Columbia University

THE CORACOID OF *SUS SCROFA*

FRANK BLAIR HANSON

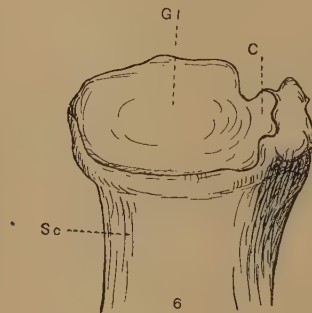
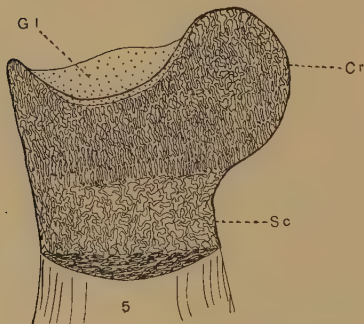
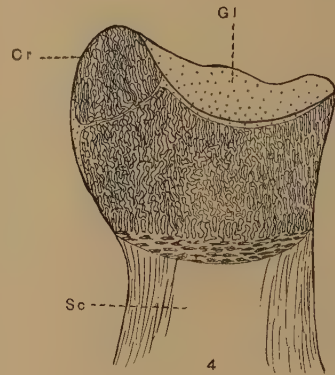
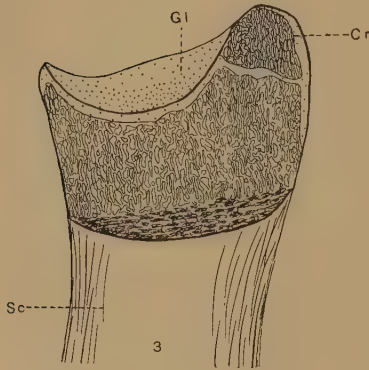
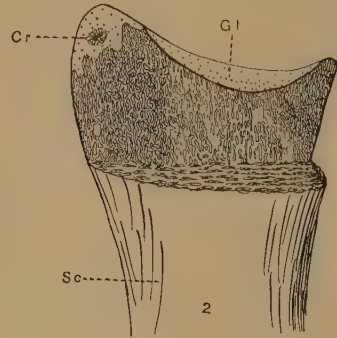
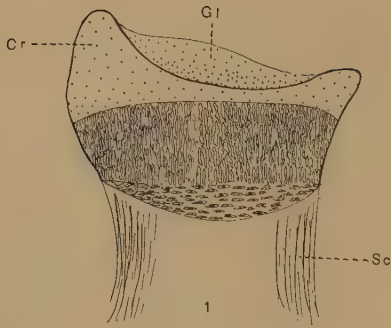
Zoological Laboratory of Washington University

SIX FIGURES

The problem of the coracoid is one of the unsolved questions of vertebrate morphology. Its homologies have been described from every possible viewpoint, yet there remains to-day as much confusion and divergence of opinion concerning this structure as in any past period.

This paper is unlike most in that it does not attempt to offer a new solution of the old problem. It is a description of the developmental stages of the subcoracoid as found in the pig. The material consists of a series of seven scapulae ranging in age from three weeks to adult life and a fairly complete series of sections of embryonic stages. No embryonic stages are figured, for in general they are not essentially different from figure 1 of the suckling pig.

Although the coracoidal part of the pig scapula is never a separate cartilage, it may be identified as early as the 26-mm. stage of the embryo as a distinct, but blunt knob, on the anterior side of the glenoid surface. This is not, however, separated from the cartilage of the glenoid, but is continuous with it, and is a constituent part of the articular surface. Following the history of this portion through close stages of the embryonic and foetal life discloses no essential change of relations or appearance until we pass from foetal to postnatal life. Figure 1 is the glenoid end of the scapula of a pig two weeks old. The relatively large cap of cartilage is one homogeneous whole, and has been so since first recognizable in the embryo. The shaft of the scapula has been cut in the median plane in an anterior-posterior direction, as have also the other stages herein illustrated. Figure 2 is the scapula of a pig three months of age. During



the interval since the first stage figure, the center of ossification for the coracoid has appeared. It lies in the center of the coracoid cartilage, and it will be noticed that the ossification of the scapula has proceeded rapidly toward the glenoid end, leaving only a thin sheet of cartilage over the articular surface and the cartilage of the coracoid surrounding its ossific center.

Figures 3 and 4 are of one-half and three-quarters grown pigs, respectively. They show the growth of the ossific center and its encroachment upon the cartilage of this region. The pig of figure 4 is approximately nine months of age, and there remains but a narrow layer of cartilage between the scapula and coracoid. The cartilage of the glenoid surface has been further reduced. Figure 5 is that of a fully matured hog. This specimen weighed 450 pounds and evidently was past what might be termed young adult life. Sections through the glenoid-coracoid region of this bone fail to show any trace of the fusion of the coracoid with the scapula. It is to all appearance one solid bone, and not knowing its early history one would not suspect that this had been two separate bones until young adult life. In figure 6 is shown the glenoid portion of a scapula from a pig one year old. It gives a good conception of the general appearance and relations of coracoid and glenoid parts. Fully one-fourth of the glenoid is composed of the coracoidal contribution.

The coracoid here described is not the coracoid process of placental mammals, but that smaller, more inconspicuous element known in man as the subcoracoid. The coracoid of the

- Fig. 1 Pig 3 weeks old. Glenoid end of scapula. No ossific center for subcoracoid. $\times 7$.
Fig. 2 Pig 3 months of age. Center of ossification present. $\times 3$.
Fig. 3 Pig one-half grown. Subcoracoid a separate bone from scapula. $\times 3$.
Fig. 4 Pig three-fourths grown. Coalescence of subcoracoid and scapula nearing completion. $\times 2$.
Fig. 5 Scapula of old boar. All trace of fusion of subcoracoid and scapula lost. $\times 1$.
Fig. 6 Young adult, one year old. Articular surface made up of three-fourths scapula and one-fourth coracoid. $\times 1$.

ABBREVIATIONS

Cr., subcoracoid *Gl.*, glenoid *Sc.*, scapula

Suidae is a so-called subcoracoid and is undoubtedly the homologue of that structure bearing the same name in man. Both are glenoid-sharing portions, and in all the details of general topography are identical.

In mammals bearing a coracoid process two centers of ossification are present—one for the coracoid process and one for the subcoracoidal, glenoid part. In the pig but one ossific center, that of the subcoracoid, is found. The pig scapula is degenerate, lacking both coracoid process and acromium.

Broom ('99) was the first to suggest that the so-called subcoracoid was merely an epiphysis, and did not enter into the coracoid problem. Gregory ('15) disputes Broom on this point and agrees with Williston that this so-called epiphysis is really the metacoracoid, and a reptilian inheritance. In a later paper, however, Gregory ('17 a) reverses this conclusion and admits that Broom may be correct and that this is merely an epiphysis. And still later in the same year, he (Gregory, '17 b) was led to believe that this subcoracoid is not merely *an* epiphysis, but *the* epiphysis of the glenoid portion of the metacoracoid or true coracoid. Gregory thus derives both coracoid process and subcoracoid from the true or posterior coracoidal element. The anterior or epicoracoidal part has been completely reduced and is not represented in placental mammals.

On the other hand, Cunningham ('16) homologizes the coracoid process with the epicoracoid, and the subcoracoid with the posterior (meta) coracoid. This is also the position taken by Lydekker ('93), Howes ('93), Williston, and others.

That Gregory may be more nearly correct than the other workers is indicated in the progressive shutting out of the epicoracoid (anterior element) from any share in the glenoid. This proceeds as we ascend higher in the scale, until in the Monotreme the epicoracoid is entirely excluded from the glenoid; in the marsupial it is only recognized as a thin cellular sheet in the early embryo which soon disappears; and no trace of it is found in the other groups of mammals unless it be, as suggested by Broom ('12), represented by the coraco-clavicular ligament.

Thus, while there has been great diversity of opinion with regard to the homology of the anterior element and the coracoid process, the main group of workers agree that the so-called subcoracoid is the homologue of the coracoidal element variously known as the true coracoid, posterior coracoid, metacoracoid, or simply the coracoid. If this be true, the coracoid in the pig may be described as a glenoid-sharing portion, which is undoubtedly the homologue of the subcoracoid of man and also the sole remaining evidence of the true or posterior coracoid which in the lower groups (birds, reptiles, amphibians) extends to and articulates with the sternum. While in most mammals this coracoid is represented by a coracoid process as well as by its epiphysis (the subcoracoid), in the pig and in the ungulates generally the resorption of the coracoid bar has been complete, leaving only the subcoracoid, which is as we would expect, since the subcoracoid has the definite function of helping to form the articular surface for the humerus.

It has been shown by Broom in various papers that in a number of marsupials the embryo or foetus has a distinct coracoid extending from the scapula to the sternum. As development proceeds a process of absorption commences in the middle of this coracoidal bar and proceeds in each direction, until, on the one hand, the entire sternal end of the embryonic coracoid disappears, while on the other, the absorption stops just short of the scapula, leaving the well-known coracoid process of the adult marsupial and of course its epiphysis, the subcoracoid.

It is not at all difficult to see how, if this process of absorption were in the pig to proceed as completely scapulaward as sternalward, that the actual condition as found in the *Suidae* would result. That this degeneration should be incomplete in most mammals, leaving a coracoid process, and complete in the pigs, therefore not leaving a coracoid process, is neither unusual nor unexpected, for degeneration processes are apt to be irregular, and, further, this condition has its exact counterpart at the sternal end of the coracoid, where in the majority of mammals no trace of the coracoid is left, yet in the rodents a coracoid process is left on the sternum, because of an incomplete de-

generation at this end. To sum up, in the Amphibia, Reptilia, Aves, and Monotremes there is a coracoid extending throughout life from the sternum to the scapula. In the Placental mammals absorption of the coracoidal bar from the middle portion in each direction results in the complete disappearance of the sternal half of the coracoid, the rodents excepted; while the scapular half of the coracoid does not completely disappear, but is represented by its distal end, the coracoid process. The Ungulates constitute an exception to this, in which the entire coracoid bar disappears, leaving no trace at either end, excepting always its epiphysis incorporated into the glenoid, and known as the subcoracoid.

CONCLUSIONS

1. The coracoid process is absent in the pig.
2. The subcoracoid is present and ossifies from a single center.
3. The subcoracoid is a glenoid-sharing portion and is the homologue of the like-named structure in man.
4. The subcoracoid has all the characteristics of an epiphysis, and may be the epiphysis of the posterior or true coracoid of the lower forms.

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Resumido por el autor, Harvey Ernest Jordan.

Estudios sobre la estructura estriada de los músculos.

IV. Discos intercalados en el músculo estriado voluntario.

El autor describe discos intercalados típicos en los músculos de la pierna del hombre, semejantes a los sencillos discos en "forma de banda" que existen en el músculo cardíaco. La presencia de tales discos en el músculo estriado voluntario está de acuerdo con la hipótesis que les supone como los representantes de bandas de contracción irreversibles que se han modificado, y, además, suministra una prueba adicional en contra de su interpretación como los límites de las células del miocardio. El autor resume y discute los cambios estructurales que sufre la fibra muscular estriada durante la contracción, los cuales se manifiestan por cambios en sus reacciones colorantes.

Translation by José F. Nonidez
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STUDIES ON STRIPED MUSCLE STRUCTURE

IV. INTERCALATED DISCS IN VOLUNTARY STRIPED MUSCLE

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ONE FIGURE

INTRODUCTION

In a series of papers¹ on the intercalated discs of cardiac muscle, the hypothesis was developed that these structures represent modified irreversible contraction bands. As stated in an earlier article (5), if this interpretation of the intercalated discs of heart muscle is correct, then we should expect to find similar structures also in skeletal muscle, under certain conditions. Continued search had, however, until recently been only rewarded by negative results. While giving the course in Histology at the College of Physicians and Surgeons, Columbia University, during the summer session of 1918, I noticed in the sections of human voluntary striped muscle (sec. no. 294) included in the students' loan sets of slides, certain peculiar structures which seemed very suggestive of the intercalated discs characteristic of cardiac muscle. Dr. George S. Huntington has very kindly given me permission to use this material for study and description. The data now available only give the information that the specimen came from a leg removed at operation by Dr. W. C. Clarke, that the tissue was fixed in formalin, imbedded in celloidin, and stained with Mallory's phosphotungstic-acid hematoxylin. The preparations are superb.

The only reference to intercalated discs in skeletal muscle which I have been able to find appears in a monograph by

¹ See bibliography in article by Jordan and Banks. *Am. Jour. Anat.*, vol. 22, p. 285, 1917.

Dietrich (1), who mentions that H. B. Schmidt demonstrated at the meeting of the Pathological Society in Erlangen in 1910 preparations of voluntary striped muscle in which occurred bands which Schmidt regarded as having the same structure as the intercalated discs of heart muscle. Dietrich, however, after careful study of Schmidt's preparations, disputes the accuracy of such an interpretation. Dietrich's description of these structures as "irregular stripes which cross the muscle bundle in successive waves," and his identification of them with the abundant contraction waves frequently seen in the cardiac muscle of human cadavers (p. 11), make it quite certain that there is no close similarity between the structures in our specimen and those in Schmidt's material. I feel the more convinced of this conclusion since certain sections through the upper third of the cat's esophagus in my collection show bands which correspond exactly to those described by Dietrich in Schmidt's sections; these bands have nothing in common in the way of essential details with those in the sections of human leg muscle. In the latter material occur definite and regular discs related to the telophragmata in a manner identical with the intercalated discs of *Limulus* and vertebrate heart muscle; in the former material occur irregular contraction waves, spanning the entire width of the tunica muscularis in the case of the esophagus and involving a variable number of sarcomeres. The discs of the leg muscle are modified single contraction bands, which apparently failed or were unable to reverse in certain sharply limited locations; the bands of the esophageal muscle are widespread contraction waves fixed either in rigor mortis or by the action of the preserving fluid.

It would be of prime interest to know the morbid conditions which necessitated the removal of the limb from which our specimen was taken. Such information might indicate the specific factors operating in the production of intercalated discs. At present we can only surmise, in accordance with our general theory regarding the formation of such discs in heart muscle, that the special conditions under which discs originate include relatively violent or prolonged tensions or exceptional strains,

such as may accompany long-continued rhythmic contraction. The correlation of such discs with specific physiologic, pathologic, and experimental conditions would seem to offer a worthwhile field for future investigations.

In order to determine whether these peculiar structures in this specimen might possibly be a common characteristic of human leg muscle, I prepared sections from three other amputated legs which had been preserved in formalin. For two of these specimens I am indebted to Dr. W. C. Clarke, of Columbia University; for the third to Dr. Stephen H. Watts, of the University of Virginia. None of these specimens contained similar discs; only occasional areas of fibers were at a midphase of contraction; the sections indicated that these muscles were for the most part in a condition of repose. In order to test the further possibility that the discs of our specimen might be the result of the special staining technic, sections of these three specimens of leg muscle were also stained with the phosphotungstic-acid hematoxylin. These preparations again gave no evidence of similar discs. In addition, pieces of striped voluntary muscle of the frog were fixed in strong Flemming's solution, in the picro-acetic solution, and in 95 per cent alcohol, and stained respectively with iron-hematoxylin and phosphotungstic-acid hematoxylin. These six sets of slides showed essentially identical conditions: the Q-disc was bisected by an H-disc of considerable width, but nothing suggestive of intercalated discs was discernible.

It is required first to establish the homology between these discs in our specimen of leg muscle and those of cardiac muscle, vertebrate and *Limulus*. This is a relatively easy matter. The description of these discs will show a detailed similarity to cardiac intercalated discs amounting practically to a morphologic identity. While it is of much theoretical interest to have discovered genuine intercalated discs in skeletal muscle, and while the discovery adds support to our interpretation of these discs in cardiac muscle as modified irreversible contraction bands, it is clearly recognized that it does not prove that the theory is entirely correct. But a prediction made on the basis of this theory has now been fulfilled. The theory, however, involves

the whole question of the mechanism of muscular contraction, concerning which there is much diversity of opinion. This fact necessitates an attempt to formulate a theory of contraction which can embrace consistently the recorded morphologic data relative to striped muscle in the various phases of contraction and extension. It is recognized also that our theory concerning the intercalated discs of heart muscle has won only limited and generally qualified acceptance. Very recent revisions of text-books of general anatomy still describe heart muscle as composed of distinct cells, interpret the intercalated discs as cement lines, and ignore the histologic evidence that during contraction and extension some substance moves within the myofibril from mesophragma to telophragma, and in the reverse direction, respectively, as demonstrated by various staining methods. That the intercalated discs in cardiac muscle are not cement lines or cell boundaries is definitely proved by the fact that they do not extend completely through a fiber in the manner of a cell membrane, but are more or less deep peripheral structures. They do not extend centrally beyond the innermost myofibrils in cardiac muscle, and an occasional disc lies superjacent to a nucleus. The relative sparsity of typical intercalated discs in the heart of *Limulus* also contravenes any interpretation of adult cardiac muscle in terms of discrete cells.

Since our theory interprets intercalated discs of striped muscle as modified irreversible contraction bands, it is required that the precise morphologic features of contraction, which serve as the basis of the theory, be definitely established. It may suffice at this point to state that by 'contraction band' we mean the deeply staining area which appears on either side of the telophragma during muscle contraction (in contrast with a similar area on either side of the mesophragma when the muscle is in repose), as first clearly illustrated by Rollet (8) in his figure of the striped muscle of *Cassida equestris* (fig. 126, Jordan and Ferguson's Text-book of Histology). By 'contraction wave,' as seen, for example, in our specimen of the cat's esophagus, we understand a much wider irregular condensed area of adjacent groups of fibers; such 'waves' in any one limited area may in-

clude many 'contraction bands.' In Rollet's figure the 'wave' would include eight 'bands.' An intercalated' disc is in its simplest condition (ontogenetically and phylogenetically) fundamentally identical, structurally and tinctorially, with such a contraction band. In mature heart muscle it has become secondarily modified through the influence of mechanical and probably chemical factors, and the increase of tissue fluid among the elements. Certain pathologic conditions (e.g., hypertrophy and atrophy) are characterized by specific varieties of discs.

DESCRIPTION

All of the fibers of the entire section, 15 mm. by 5 mm. in extent, have a fairly uniform structure. A deeply staining Q-disc alternates regularly with a lightly staining J-disc. These two discs are of approximately equal thickness. The Q-disc is bisected by a lightly staining H-disc. The resulting subdivisions of the Q-disc have in general approximately the same thickness as the dividing H-disc. However, there is some variation in the thickness of the H-disc in different fibers. The J-disc is bisected by a distinct membrane, the telophragma (fig. 1, *T*). A mesophragma (M-membrane) is only discernible in occasional small areas of several of the fibers. The Q-disc is colored dark purple; the J and H discs stain a light pink color; the telophragma takes a light purple stain. This description could apply equally well to a stretched fiber or to one at midphase of contraction. The available data are not sufficiently precise to warrant a final conclusion regarding the actual functional condition of the fiber, that is, whether contracting or passing into repose or stretched. All things considered, however, I incline to regard the muscle as fixed in a slightly stretched condition.

The illustration shows three intercalated discs—one isolated, two arranged in a pair. Very rarely a group of three or even four successive discs occurs. Nowhere in this section, barring groups of three or four discs, are the discs more numerous than indicated in this illustration; in general they are less closely spaced, the distribution varying in different portions of the section.

The discs consist of a row of bacillary elements in close latera-juxtaposition, joined together through the middle by a telol phragma. These bacillary elements are portions of the included myofibrillae. The interbacillary tissue fluid takes a slightly

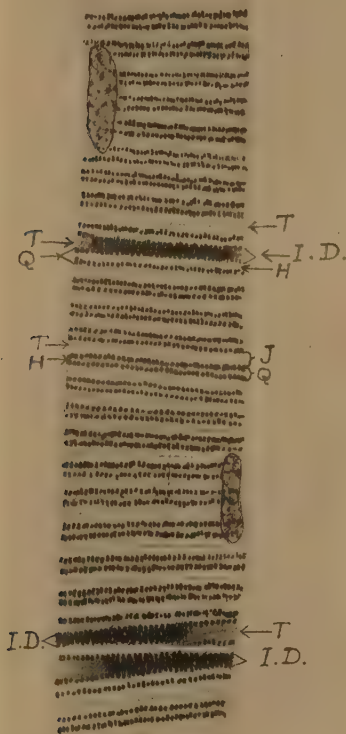


Fig. 1 Drawing of portion of longitudinal section of voluntary striped muscle fiber of human leg (formalin fixation, Mallory's phosphotungstic-acid-hematoxylin stain). *T*, telol phragma (ground membrane, Z-line); *J*, clear (isotropic) disc; *Q*, dark-staining (anisotropic) disc; *H*, Hensen's disc; *I.D.*, intercalated disc. *T* stains lightly purple, *Q* and *I.D.* deeply purple, *J* and *H* light pink. The portion between the two nuclei was drawn by aid of a camera lucida; the portion below the lower nucleus, including the pair of intercalated discs, was added free hand from an adjacent fiber. Magnification 1500 diameters.

deeper stain than the substance of the H-discs or the uninvolved J-discs. Taken as a whole the discs resemble a double-comb structure, the 'bar' of the 'comb' being formed by the telophragma. The discs are more or less deeply placed peripheral structures. Careful focusing reveals the fact that they do not completely span the fiber. The isolated disc is typical; it is less dense laterally, showing thus the bisecting telophragma; a few discs are of equal and maximum density throughout.

Many of the discs are in the condition illustrated in the lower pair of discs; that is, drawn out for some distance on one side, this condition generally being confined to alternate sides of the discs in a pair. These discs are comparable to 'contraction bands' as defined above; as such they might be in late phases of contraction or early phases of extension, that is, in incomplete contraction or extension; or they might be contraction bands drawn out laterally by a stretching of the fibers. The stretching of the discs may have been the result of the relatively greater contraction on the part of adjacent portions of the unmodified fibers during fixation or normal function. If these 'discs' actually represented unmodified contraction bands, then we should expect to find them more abundantly and more extensively arranged in groups, the latter in lateral alignment in the form of contraction waves. In a contracting or relaxing muscle we should not expect to find the bands so generally isolated and so sharply localized. In view of these facts, it seems more likely that the discs have become modified through tension and that perhaps the entire fiber was in a stretched condition. Moreover, the fibers are frequently distorted, that is, sharply bent or drawn out in the vicinity of the discs, facts which indicate that the discs are levels of relative weakness.

In my earlier studies of the intercalated discs of cardiac muscle I have shown that the simplest type of disc, both ontogenetically and phylogenetically, is a band form bisected by the telophragma. Primary variations of this original type produce discs that are bounded on one side by the telophragma, and such as are bounded on both sides by telophragmata. Further secondary modifications resulting from the operation of mechanical

factors, including chiefly oblique tensions in consequence of the branched condition of the fibers of the heart musculature, lead to the various terraced forms. Hypertrophied muscle, characterized by longitudinally splitting myofibrillae, contains only serrated types. The discs of our specimen of leg muscle correspond, then, to the original band type with bisecting telophragma. In accordance with my hypothesis that intercalated discs are modified irreversible contraction bands, the discs bounded on only one side are interpreted as contraction bands only half of which became irreversible; those bounded on both sides by a telophragma as fused adjacent irreversible halves of successive contraction bands.

Viewing the fiber of the illustration as in midphase of contraction, we may more closely examine the isolated disc. The condition of this portion of the fiber may be indicated by the following formula: $T + J/2 + Q/2 + H + \text{Disc} (Q/2 + T + Q/2) + H + Q/2 + J/2 + T = 2 \text{ sarcomeres}$. In other words, the disc includes adjacent halves of successive Q-portions fused along the telophragma. The structure in question corresponds to a contraction band and to a simple type of intercalated disc. As such it consists of deeply staining, laterally aligned, portions of adjacent myofibrils bisected by a telophragma. Since these contraction bands are apparently irreversible as indicated by their localized distribution in the absence of contraction waves, and slightly modified as indicated by the cloudy appearance of the lateral 'stretched' portions, they would seem to be typical intercalated discs. As shown in a previous paper (6), the original discs (irreversible contraction bands) are modified in part also by the accumulation of tissue fluid in the intervals of the adjacent portion of the myofibrils as indicated by the considerable precipitation of silver nitrate in these locations. The occurrence of typical intercalated discs in voluntary striped muscle, as here described, fulfills the prediction made by deduction from the hypothesis constructed from the histologic data derived from the study of cardiac muscle, and to this extent it gives support to this hypothesis.

DISCUSSION

The above-outlined interpretation of the discs as modified irreversible contraction bands calls for a brief discussion of the histologic features of contraction. The histologic evidence shows that contraction and extension are accompanied by the passage of a deeply staining substance from the mesophragma to the telophragma and vice versa, respectively. This statement calls for evidence of the existence of a mesophragma in striped muscle. It must be admitted that such a membrane is under usual conditions not discernible with our present means of microscopic observation. In an earlier paper (4) on the leg muscle of the sea-spider, I showed that a mesophragma becomes discernible in the contracting portion of a fiber which has at the same time become stretched, because of the already fully contracted condition of one end of the fiber. Assuming that the mesophragma is too delicate to come within the limits of visibility under ordinary conditions, stretching of the fiber would result in lengthening the sarcomeres and in reducing their diameter, thus causing a relaxation of lateral tension and permitting the mesophragma to coarsen to a point where it comes within the limits of microscopic vision. This is in essence the idea of Heidenhain (3), who claims to be able to see a mesophragma even in human cardiac muscle. The behavior of the lightly stained portion of the Q-disc (i.e., the transient H-disc) under conditions of contraction and stretching points also to the presence of a dividing mesophragma in the Q-disc. The deeply staining portion of the Q-disc under these conditions divides exactly along a middle plane; such precise division of this disc could hardly occur so uniformly unless the disc were bisected by a true membrane. A small portion of at least one fiber of this section also shows a mesophragma with great clearness. This fiber is at midphase of contraction and the portion showing the mesophragma is apparently under some tension. That the mesophragma of a sarcomere, however, differs to some extent from the terminal telophragmata is indicated by the facts that festoons are not formed in the sarcolemma at its levels, the

myofibrils are not bent along its levels in distorted fibers, and the nuclear wall is not drawn out into ridges at its levels. This would seem to indicate that the mesophragma, in contrast to the telophragma, is not attached to sarcolemma, myofibrils and nuclear membrane. However, the essential difference between these two membranes may be simply one of relative strength or rigidity. The mesophragma may be sufficiently elastic to be able to yield without rupture to distorting forces, or it may be too delicate to withstand the strains that operate in fixed tissues to demonstrate the relationship between the telophragma and the myofibrils, sarcolemma and nuclear membrane.

It would seem, therefore, that any discussion of muscle contraction may confidently proceed upon the basis of the existence of two membranes, the terminal telophragmata of the sarcomere and the bisecting mesophragma. Since Merkel (7) first called attention to the 'reversion of the striae' during contraction, this phenomenon has been repeatedly described in many different specimens of striped muscle. It was especially well shown in the illustration of Rollet (8). Englemann (2) criticised this interpretation on the basis of his demonstration that, as revealed by the polariscope, the anisotropic substance of the Q-disc does not move during contraction, but remains permanently segregated more or less closely about the mesophragma. This conclusion is supported by the observations of Schaefer (9) on muscle treated with Rollet's gold chloride method.² Englemann (2) would seem to interpret the phenomenon of an apparent reversal of striae, as shown in stained sections, on the assumption that the absorption of the isotropic substance of the J-disc by the anisotropic substance of the Q-disc (upon which contraction is supposed to depend) causes a relative condensation of the sarcoplasm about the telophragma and thus produces a relatively deeper-staining area in this location. Schaefer (9), however, attributes the phenomenon to "the deeply moniliform

² It is now clear to me, however, that the sarcostyle of the wasp's wing muscle which Schaefer regards as in the contracted condition is one swollen and shortened by the action of the hypotonic formic-acid-water solution used in Rollet's technic.

shape of the fibrils" (p. 184), which he believes "tends to cause the constricted parts to appear dark." In other words, Schaefer interprets the apparent reversal of the striae during contraction as an optical illusion. He states further that "In alcohol preparations (both of the wing muscles and of the ordinary or leg muscle) in which the sarcous elements have been stained, there is no appearance of reversal of striation; the darkly colored sarcous element always occupies the central or bulged part of the sarcomere, and the unstained hyaline substance occupies the constricted parts of the sarcostyle" (p. 185). The latter statements seem to me in direct contradiction to the facts. In alcohol-fixed tissue stained with iron hematoxylin the striae do reverse during contraction, the stainable substance of the Q-disc moving towards the telophragma; and the deeply staining discs of contracted muscle, that is, the 'contraction bands,' are bisected by the telophragma, where the constriction, if any occurs, is uniformly located in contrast with the deeply staining band of extended muscle which is bisected by the mesophragma.

Furthermore, that the movement of material during contraction is not solely from the isotropic to the anisotropic substance, that is, from the telophragma to the mesophragma, but largely in the reverse direction is shown by the fact that the median portion of the Q-disc, the portion farthest removed from the isotropic substance, becomes pale first; this gradual paling or loss of tingibility passes distally in opposite directions from the mesophragma towards the telophragmata, and the myofibrils become swollen distally and assume a knobbed appearance as if material were actually accumulating terminally in the sarcomeres. These knobbed ends stain even more deeply than the general Q-substance of the relaxed fiber. This phenomenon was especially well shown in the sea-spider muscle and has been illustrated by various investigators on the histology of contracting muscle. If contraction only involved an absorption of isotropic by anisotropic substance, by reason of which the striae apparently reversed due to a change of relative density of the J and Q discs, then the terminal portion of the original Q-disc

should become pale first instead of the farthest removed portions (from the nearest point of alleged absorption) or the middle portion of the Q-disc.

To summarize, these are the fundamental histologic data upon which an adequate theory of muscle contraction must be based or with which it must harmonize, and which it must consistently embrace. The occurrence of a mesophragma and a telophragma as constituent elements of a sarcomere, the telophragma at least being intimately connected with the myofibrils, the sarcolemma, and, where a nucleus intervenes, with the nuclear membrane; changes, as evidenced by an alteration in staining capacity, within the myofibrils, these changes involving a movement of a deeply staining substance from the mesophragma to the telophragma, apparently without change in position of the anisotropic granules of the original Q-discs; these changes produce a 'contraction band,' that is, a deeply staining 'disc' bisected by a telophragma; such discs, failing to 'reverse' locally, correspond to the simplest type of intercalated discs of cardiac muscle, and may become further modified (physically, chemically and morphologically, involving the accumulation of tissue fluid) to form genuine intercalated discs; the appearance of contraction bands is coincident with a shortening and thickening of the myofibrils and of the muscle fibers and of the muscle as a whole; contraction and mechanical tension produce a comparable effect upon the Q-disc, a separation along the level of the mesophragma; when constrictions supervene in a contracted fiber they are at the levels of the contraction bands, that is, at the levels of the deeply staining portions, and are due at least in part to the fact that here the bisecting telophragma is attached to the sarcolemma, between two successive points of attachment to which the sarcolemma is festooned permitting thus a bulging of the intervening sarcous substance at this level.

The above-detailed facts are not in conflict with the newer theories of muscle contraction that attempt to explain contraction in terms of surface tension or electrical phenomena among the ultramicroscopic sarcous particles, in so far as these theories do not postulate an absorption of the isotropic sub-

stance of the J-disc by the anisotropic substance of the Q-disc or a passage of substance from the region of the telophragma towards the mesophragma. Both the change in structure and in staining reaction of the involved portions of the myofibrils demonstrate that the movement of material is largely in the reverse direction during contraction, from the mesophragma towards the telophragma. Such movement is coincident with contraction. How these two concurrent phenomena are fundamentally or causally related is a question whose answer lies outside the scope of the present investigation.

The chief aim of this investigation, finally, concerns a demonstration that genuine intercalated discs occur in voluntary striped muscle under certain conditions, and the further support of our hypothesis that the intercalated discs of cardiac muscle are in essence modified irreversible contraction bands. If the conclusion that these discs of human leg muscle are comparable with the intercalated discs of heart muscle is correct, as seems incontrovertible, then the latter discs can for an additional reason be no longer regarded as cell boundaries.

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Resumido por el autor, Harvey Ernest Jordan.

Estudios sobre la estructura del músculo estriado.

V. Histología comparada de los músculos de las patas y alas de Mantis, con especial mención de los discos en N y los sarcosomas.

El disco en N es una estructura bien manifiesta en el músculo de las patas, pero no puede discernirse en el del ala. Está formado por elementos intrafibrilares y contribuye a la formación de la banda de contracción. Una banda de contracción comprende la substancia intensamente coloreable de las mitades opuestas de dos discos anisótropos sucesivos, los dos discos en N intermedarios y el telofragma bisector. Los sarcosomas son cuerpos intrafibrilares (intersarcostílicos), de naturaleza lipoide en su mayor parte. Se agregan en ciertos estados de contracción formando grupos en forma de J y Q. Tanto los discos N como las bandas de contracción son esencialmente independientes de los sarcosomas. El telofragma actúa como una barrera efectiva que se opone al paso de los sarcosomas. Tal barrera no existe en la región ocupada comunmente por el mesofragma. En el músculo de las alas o de las patas de Mantis no existe mesofragma. El autor discute la relación de los sarcosomas con las mitocondrias. Las miofibrillas se continúan directamente con las fibrillas tendinosas.

Translation by José F. Nonidez
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STUDIES ON STRIPED MUSCLE STRUCTURE

V. THE COMPARATIVE HISTOLOGY OF THE LEG AND WING MUSCLES OF THE MANTIS, WITH SPECIAL REFERENCE TO THE N-DISCS AND THE SARCOSOMES

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THREE PLATES (THIRTY FIGURES)

The wing muscle of the mantis furnishes an exceptionally favorable material for the investigation of the interfibrillar sarcoplasmic granules, or 'sarcosomes,' characteristic of insect wing muscle. They are here unusually large and abundant. Since they change their shape and position during contraction of the muscle fiber and since they have been associated with the accessory disc or N-stripe of complexly striped insect muscle, their study involves the phenomena of muscle contraction both in wing and leg muscle.

The material is of the species *Stagmomantis carolina*. It includes both young and adult individuals. For some of the adult specimens I am indebted to Mr. Massie Page. These were variously fixed in strong Flemming's fluid, a 10 per cent formalin solution, and in 95 per cent alcohol. The sections were cut in paraffin at 5 μ , and stained with iron-hematoxylin. Some were counterstained with the picric-acid-fuchsin combination. Some of the formalin-fixed sections were stained with Mallory's phosphotungstic-acid hematoxylin. Teased fresh tissue mounted in glycerin was also used. For a young, newly moulted specimen, 38 mm. in length, I am indebted to Prof. W. A. Kepner. The latter material was fixed in aceto-sublimite and stained with iron-hematoxylin. It is especially valuable by reason of the clear data it supplies with regard to the manner of muscle-tendon connection.

LEG MUSCLE

The description of the comparative structure of the wing and leg muscles begins most conveniently with the latter variety, since sarcosomes do not occur here to obscure the several striations in their alterations during contraction. As will appear below, leg and wing muscles, aside from the presence of numerous interfibrillar sarcosomes in the latter have an essentially identical microscopic structure.

In the extended or relaxed condition, the leg-muscle fiber presents an alternation of light and dark discs, the former approximately twice the width of the latter (fig. 1). The telophragma is distinctly seen as a deep-staining granular membrane, bisecting the lighter disc. The granules of the telophragma are swellings at the points of attachment of the myofibrils. That the connection between the myofibrils and the telophragmata is very intimate is demonstrated by conditions in distorted fibers, where the fibrils are held rigidly in place along telophragma levels regardless of modifications in the normal relations of the myofibrils within the sarcomeres. The conditions here are practically identical with those originally described for the skeletal muscle of *Limulus*.¹⁰

The fiber is enveloped by a robust sarcolemma, frequently festooned between, and firmly united to, the telophragmata (fig. 6). The nuclei are peripherally located (figs. 4 and 6). Their number is increased relatively towards the point of insertion into the exoskeleton. In transverse section the fibers have a generally polygonal outline. The myofibrils are strap-like peripherally; centrally they have a cylindric form; many of the peripheral fibrils show a radial split, indicating longitudinal division. There is no suggestion of a mesophragma at any stage in the contraction process.

Certain fibers with the same general features as those described for the extended or resting fiber present an additional striation on either side of the telophragma, namely, an accessory disc of Merkel or the so-called N-stripe (fig. 2). As the fibers enter contraction, the dark or Q-disc becomes bisected

by the appearance of the H-disc, as illustrated in figure 3. This figure shows also the N-disc; in the lower portion of this figure, Q and N are seen in process of fusion. A subsequent stage is illustrated in figure 4. Here Q and N have fused throughout, and the resulting composite dark discs are approaching the telophragmata. The union of two such composite discs with an intervening telophragma produces a contraction band (fig. 5). Figure 6 is at approximately the stage illustrated in figure 5. The difference in appearance is due to a difference in degree of destaining. Figure 7 also corresponds to the stage shown in figure 5; it differs in appearance somewhat from the fiber of figure 5 probably by reason of having become stretched. In figure 8 is illustrated a completely contracted fiber, in which deep-staining contraction bands alternate with light-staining intermediate bands of approximately equal thickness. The foregoing description shows that a contraction band consists of the fused opposite halves of two consecutive Q-discs, plus the two intervening N-discs and the included telophragma. It shows, moreover, that a deep-staining substance of the Q-disc of the fiber in repose actually moves toward the telophragma during contraction.

WING MUSCLE

As can be seen from figures 9, 10, 11, 12 and 13, the wing muscle during contraction presents the same alterations in the striations as the leg muscle. However, nothing strictly comparable to the N-disc of leg muscle seems to occur in wing muscle. Figure 11 illustrates especially well the passage of material from Q to the telophragma.¹ The fibers are relatively enormous structures, irregularly polygonal in outline in transverse section (fig. 23).

¹The slightly greater length of the sarcomeres of certain contracting fibers, as compared with those of the resting fibers, frequently seen in histologic preparations, may be explained as the result of the superposition of a condition of stretching (figs. 1, 3 and 8; and figs. 9, 11 and 13). It would seem that those portions of a fiber which are at midphase of contraction are more responsive than those at rest to the tensions incident to the death and fixation of contracting fibers where one end is in full contraction (see paper on leg muscle of sea-spider²).

The nuclei are generally peripherally located (figs. 21 and 22), but occasional nuclei lie in the region between the center and the periphery (figs. 23 and 24). The peripheral myofibrils have the form of broad lamellae, those more centrally placed are cylindric in form. Adjacent fibers differ considerably with respect to the form and arrangement of the myofibrils. (Compare figs. 22, 23, and 24.)

The feature of greatest interest about the wing muscle pertains to the interfibrillar granules or sarcosomes (figs. 14 to 20). These are preserved about equally well in the formalin- and the Flemming-fixed tissues. In alcohol-fixed tissue they cannot be discerned. Alcohol destroys the bodies, leaving a granular débris. Their reaction to the several fixing fluids suggests that they are largely lipoid in chemical constitution. Since they cannot be seen in formalin-fixed leg muscle, I conclude that sarcosomes do not occur in this leg muscle. In the wing muscle the sarcosomes are apparently scattered at random in the interfibrillar sarcoplasm, including the peripheral and perinuclear regions (figs. 23 and 24). They are apparently unconnected with the myofibrils and disconnected among themselves, for in teased fresh material they readily separate out from among the fibrils.

In the sectioned material the sarcosomes, especially the larger, have an oval shape; in the isolated condition in teased fresh material they are spherical in shape. These observations indicate that the sarcosomes have a semifluid consistency, their oval shape when confined within the fiber being due to compression between adjacent muscle columns. In certain portions of the fixed tissue the sarcosomes appear to consist of hollow vesicles (fig. 15), only a robust shell ('membrane') persisting. Under these conditions, the sarcosome 'negatives' are more nearly spherical in shape. The adjacent sarcostyles are curved around these vesicles, and are firmly held in place by the telophragmata. Moreover, in almost any area, sarcosomes of different consistency can be seen; some stain deeply, others only lightly. In general, the smaller stain more deeply. The vesicular sarcosomes are generally spherical in form (fig. 14). These observations indicate that the sarcosomes undergo changes in

physical and chemical constitution, and suggest their transient character. As regards the morphologic changes during contraction, the sarcosomes appear to have a passive rôle.

Figures 17 and 18 represent fibers (destained to a point where the Q-disc no longer shows) in the condition of repose or extension. Here the granules are aggregated within the region of the Q-disc. The majority have an oval form, the long axis of the granule being parallel with the long axis of the fiber. Figure 19 represents a fiber at about midphase of contraction, comparable with the more deeply stained fiber of figure 11 and the leg fiber of figure 4. Here the smaller spherical and more deeply staining granules have become aggregated along the telophragmata. Figure 20 represents a fiber in practically complete contraction. The destaining has been carried to a degree which no longer leaves the contraction band conspicuous. The fiber corresponds to a stage close to those illustrated in figures 12 and 13. The most interesting fact relates to the altered shape of the sarcosomes; these elements are still oval in shape, but in a fiber in this condition the long axis of the oval granule is placed at right angles to the long axis of the fiber. This alteration in the axes of the oval granules is presumably due to pressure exerted by reason of the closer apposition of the successive telophragmata in the contracted fiber. Similar mechanical factors no doubt operate in the production of the occasional angular, cup-form, collapsed and the other irregular types of sarcosomes.

The foregoing description of the movement of the sarcosomes during muscle function discloses two other important facts: 1, The effective confining capacity of the telophragmata and, 2, the ineffective confining capacity or absence of mesophragmata. The sarcosomes apparently cannot pass through the telophragmata, and there is apparently nothing to prevent their passage through the area occupied by a mesophragma in certain other insect muscle fibers.

A matter which presents great difficulty of explanation is the considerable variation in density of closely adjacent fibers. A comparison of figures 22, 23, and 24 and of figures 14 and 20

will give some idea of this variation. In figure 22 the lamellar sarcostyles are of very irregular form; in figure 23 the lamellar sarcostyles are quite regular in form and are numerically greatly preponderant; in figure 24 only a narrow peripheral layer is composed of only relatively delicate lamellae, the cylindric type greatly preponderating. In figure 14 the lamellae are widely separated and the telophragmata are ruptured; in figure 20 the telophragmata are all intact and hold the myofibrillae firmly in place. Figures 14 and 24 are in similar condition, also figures 20 and 23. At first sight the difference between such fibers as are illustrated in figures 14 and 20, that is loose, and compact types, might seem to be due to a relative abundance of sarcosomes. Careful microscopic examination, however, makes it clear that the sarcosomes are about equally numerous in both types. The difference in general appearance is due primarily to a rupture of the telophragmata in some fibers, permitting a separation of the myofibrils and thus bringing into sharper view the included sarcosomes. This matter then resolves itself into the question as to why the telophragmata are ruptured in certain fibers. Since intact fibers occur in all phases of contraction, a condition like that illustrated in figure 14 is not related to a specific functional stage. (Compare figs. 11, 17, 18, 19, and 20.) Nor is the condition characteristic of smaller fibers or of the central portion of fibers, as might perhaps be inferred from figures 23 and 24. While the looser and more compact fibers generally occur in separate groups, such segregation is by no means invariable. Occasionally a loose fiber may lie among a group of compact fibers or a compact fiber may become loose toward one end in the section.

These observations suggest that the ruptured telophragmata are not fixation artifacts. But the data are not sufficiently precise to warrant a final conclusion. The condition might possibly represent a disintegration of certain fibers. But the normal condition of the sarcosomes (compare figures 14, 15 and 19) would seem to be conclusive evidence against this interpretation. Whether the condition illustrated in figure 14 is a fixation artifact or a phase of regression must remain undecided

for the present. The condition, however, is of much importance from another view-point, as will be made clear in the subsequent discussion when the question of the character of the membranes, telophragmata and mesophragmata, in wing muscle will be discussed. The meaning of the difference in shape and size of the sarcosomes, and their segregation during contraction (compare figs. 11, 17, 19, and 20), will also be considered in the subsequent discussion.

Certain of the peripheral fibers which remained covered by chitin during fixation in Flemming's fluid were only poorly preserved. The lamellae appear swollen and broken and in part more or less fused into an irregular network (fig. 30). The general appearance is that of muscle tissue fixed by Meves' technic for mitochondria. Such fibers after staining with iron-hematoxylin are seen to contain filar and bacillary mitochondria in the interlamellar sarcoplasm.

MUSCLE-TENDON CONNECTION

The data regarding the mode of muscle connection to the chitinous exoskeleton are so clear and definite in this material that it seems desirable to give a brief description. I shall only describe the condition here presented without discussion or review of the pertinent literature. The literature on the subject of muscle-tendon connection has been most recently assembled in a bibliography and fully reviewed by Downey.⁴ In the mantis the muscle and tendon fibrils are continuous. A similar condition was previously described for the skeletal muscle of the sea-spider⁹ and the scorpion.¹¹ This mode of muscle-tendon connection accords with that described by O. Schultze²⁰ for vertebrate, including human skeletal muscle generally. Downey, however, claims that in the crayfish the muscle fibers end abruptly and become 'dovetailed' into the tendon, the muscle fibrils thus being non-continuous with the tendon fibrils.

In the mantis the muscle fibers, wing, abdominal and leg, are inserted directly into the chitin. In the abdomen certain muscle fibers can be followed for long distances in their passage

between the hypodermal cells, where they are still clearly striated, but apparently lack sarcosomes, to points where they separate into delicate fibrils which are inserted into the chitin. The evidence is most clearly presented in the case of the muscles related to the ovipositor in a newly moulted specimen of 38-mm. length (fig. 29). The hypodermal cells, with indistinct boundaries, here form a relatively thick layer, upon which rests young chitin. The muscle fibers show only a faint striation and contain relatively very many nuclei. Transverse sections of these fibers show that the sarcostyles are delicate lamellae (fig. 26). The nuclei at this stage multiply both by mitosis and amitosis (fig. 25), later only by amitosis. The muscle fibrils pass between the hypodermal cells into the superjacent chitin. Among the hypodermal cells the muscle fibrils become compacted into bundles. Where they pass into the chitin they spread out again in fan-like fashion before they terminate. Where they pass among the hypodermal cells, the myofibrils stain intensely black. This difference in staining reaction is a physical phenomenon rather than an indication of a chemical change, for within the chitin the fibrils again stain only lightly in a manner similar to that before they enter the hypodermis. Moreover, in certain regions the hypodermal fibril bundles stain lightly and so appear no different from their condition before entering the hypoderm and after passing beyond it. Acid-fuchsin counterstain reveals no sharp difference between the myofibrils before and after they enter the hypodermis. The fibrils among the hypodermal cells and within the chitin are actually muscle fibrils which have lost their cross-striated condition. In this sense, that is, calling the modified muscle fibrils within the hypoderm and chitin 'tendon,' muscle fibrils and tendon fibrils are strictly continuous. Below the hypodermis occurs a more or less complete connective-tissue layer, forming a loose fibrillar and nucleated 'basement membrane' for the hypodermal cells.

DISCUSSION

The lamellar type of sarcostyle is characteristic of arthropod muscle.² A question of much theoretical importance concerns the intimate structure of this element. Is the lamella the ultimate morphologic unit or is it a composite structure? In an attempt to answer this question, I dissected fresh wing muscle of the mantis in diluted glycerin. Lamellae could readily be separated from their neighbors within the fiber. Such lamellae were easily divided into coarser and finer fibrils. In transverse sections of fixed and stained material certain lamellae appear perfectly homogeneous; others seem to contain constituent fibrils. In longitudinal sections also certain lamellae seem to contain a central more condensed and deeper-staining core or fibril. These observations suggest that the lamellae include fibrils which are imbedded in a homogeneous lamellar sarcoplasm. The histogenetic process gives confirmatory data. The young fibers (fig. 26) contain delicate lamellae and cylindric fibrils. At later stages the fibrils are largely coarse lamellae, some of which may be seen splitting longitudinally (fig. 27). This process of myofibril increase by longitudinal splitting, radial and tangential, still obtains in adult fibers (figs. 23 and 24). The complete process follows this order: The first formed myofibrils are cylindric; subsequently appear lamellae; these latter produce other lamellae and central cylindric fibrils by longitudinal fission. In adult fibers some of the lamellar sarcostyles instead of splitting off cylindric myofibrils retain them within their substance, at least for a time, thus producing composite myofibrils in the form of lamellae. The evidence, then, indicates that the lamellar sarcostyles contain ultimate genuine myofibrils. The condition of the fiber at its hypodermal terminal, where it becomes resolved into fine fibrils which pass in groups between the hypodermal cells to their attachment with the chitin, also demonstrates the essential composite fibrillar nature of the lamellar sarcostyles.

² The wing muscles of certain insects, e.g., diptera, hymenoptera and coleoptera, present striking exceptions.

The similarity between the adult structure of insect muscle and the embryonic structure of vertebrate muscle is a fact of much interest. We have here a specially beautiful illustration of the law of biogenesis ('recapitulation theory'). For the purpose of more clearly presenting this point I have added a figure of a developing muscle fiber of the newly hatched rainbow trout (fig. 28). As can be seen by comparing figures 23 and 28, the structural similarity is very close. As in the adult wing muscle of the mantis, the skeletal muscle fiber of the trout embryo contains peripheral lamellar and central columnar sarcostyles. The latter are derived from the former by central tangential splitting. The peripheral lamellae split also radially into daughter lamellae. At an earlier stage the muscle cell of the trout embryo contains a single, deeply staining primitive myofibril lying close against the nuclear wall. The genesis of the original myofibril could not be determined. Heidenhain⁶ has recorded similar observations on trout-embryo muscle, but he also was unable to determine the origin of this initial myofibril. The subsequent history, however, is clear. The original myofibril (primitive sarcostyle) splits radially into four primitive lamellae, each one of which again splits longitudinally, the repetition of this process leading to a condition illustrated in figure 28. Later steps in the continuous longitudinal splitting (radial and tangential) lead to the adult condition of vertebrate striped muscle with its definitive structure of ultimate myofibrils (definitive sarcostyles) collected into Kölliker's columns (Cohnheim's areas in transverse section). This line of evidence suggests that the lamella of the insect muscle corresponds rather to a Kölliker's column of vertebrate muscle than to an ultimate fibril or genuine sarcostyle. And, as stated above, certain lamellae clearly reveal included fibrils. In the mantis material, accordingly, the term 'sarcostyle' is strictly synonymous neither with 'myofibril' or 'Kölliker's column,' as these terms are used in connection with adult vertebrate skeletal muscle. The myofibrils of mantis correspond to the sarcostyles (synonymous with myofibril) of vertebrate skeletal muscle; the sarcostyle of mantis consists of a collection of myofibrils in the shape of a

lamella or a cylinder. Groups of such sarcostyles correspond to a Kölliker's column of vertebrate muscle. The sarcosomes are therefore strictly intersarcostylic in distribution.

Thulin²¹ has recorded the observation that the wing muscles of certain insects (coleoptera, hymenoptera, and diptera), and the analogous pectoral muscles of birds and bats, lack both the telophragmata and the mesophragmata. Holmgren⁸ likewise believes that telophragmata are lacking in the wing muscle of certain insects. His illustrations of the wing muscle of *Libellula*,⁷ however, show these membranes very conspicuously. A comparison of Thulin's illustrations of the wing muscle of the wasp and of the bumblebee with the several types of fibers in the mantis wing muscle, shows that his material corresponds with the looser variety of mantis muscle. Certainly, the compact wing-muscle fibers of mantis contain definite and conspicuous and perfectly typical telophragmata. The suggestion presents itself that possibly Thulin had conditions for the basis of his description like those represented in figure 14 where the telophragmata had become ruptured and had apparently very generally disappeared by reason of a parallel orientation of the fragments with the myofibrils. Absence of telophragmata in these muscle fibers would therefore seem to be secondary to changes, either artificial or such as are related to regressive processes, in the fibers resulting in a rupture of those membranes.

As regards the mesophragmata, however, the case is different. I have previously shown that in the leg muscle of the sea-spider a mesophragma comes into view under certain conditions.⁹ These conditions include a stretching superimposed upon a midphase of contraction. A contracting fiber which is firmly attached to the chitin at both ends may become modified in this way. One end may be in full contraction while the middle portion is still at midphase. This middle is then put under tension, the diameter of the fiber is reduced, and the mesophragmata, which under ordinary conditions are beyond the limits of microscopic vision coarsen and are brought within visual limits. The presence of an H-disc at midphase is also a factor in rendering the mesophragma more conspicuous. These ob-

servations would seem to confirm Heidenhain's⁵ claim that a mesophragma is universally present in striped muscle, but may be invisible by reason of its extreme tenuity. Such explanation may apply also to the apparent absence of a mesophragma in the leg muscle of the mantis.

However, in the wing muscle of the mantis a mesophragma seems actually to be lacking. The only plausible alternative interpretation would be one postulating a fenestrated condition of this membrane, permitting a free passage of the sarcosomes. For while the sarcosomes are effectively barred in their movements by the telophragma, no such barrier occurs in the region where the mesophragma, when present, is located. On the other hand, the fact that the deeply staining portion of the Q-disc of the wing-muscle fiber divides equally during contraction, and the halves move in opposite directions toward the telophragmata, would seem to demonstrate the presence of some sort of membrane sufficiently complete to initiate the division of the Q-substance at certain definite and regular levels. The evidence, then, seems to indicate that if a mesophragma is actually present it must be fenestrated, or at least be of such a nature as to be unable to offer a barrier to the free movement of the sarcosomes. If the evidence is adjudged to indicate the absence of a mesophragma, the equal division and opposite movement of the Q-substance during contraction, and in a stretched fiber, present a problem for which no very plausible explanation is at present at hand. In this case we would seem compelled to postulate a membranous partition in the Q-substance of the sarcostyle which has no representative, contrary to the case of the telophragma, in the intersarcostylic regions.

The foregoing discussion leads logically to a consideration of the phenomena of contraction. Englemann and Schaefer¹⁸ assume a movement during contraction of fluid from the isotropic J-disc to the anisotropic Q-disc. Contraction is explained as depending upon a shortening and widening of the sarcomeres following a swelling of the anisotropic granules of the Q-disc by reason of absorption of fluid from the J-disc. Englemann has demonstrated by means of the polariscope that the aniso-

tropic granules do not change their location during contraction (cited from Schaefer). The apparent reversal of striations following contraction, first described by Merkel and most clearly illustrated by Rollet,¹⁶ is believed by Englemann and Schaefer to be an optical illusion due to the movement of fluid from J to Q, producing a condensation of the former disc and a rarefaction of the latter. Granting that the anisotropic granules retain their original segregation in Q during contraction, and even that fluid may pass from J to Q, there still remains the fact of an additional movement of some deeply staining constituent of Q to the telophragma of J, which is the chief factor in the formation of the contraction band as seen in stained sections, and which effects a true reversal of striations during contraction with respect to this deeply staining constituent of the intrafibrillar sarcoplasm. This is clearly shown in figure 11. The Q-portions of the adjacent myofibrils lengthen, at the same time becoming lighter-staining and more slender medially, and deeper-staining and knobbed terminally. This terminal knobbed condition of the Q-segments of the myofibrils during contraction demonstrates that some substance actually moves towards the z-membrane. If the only movement of substances concerned in contraction were from J to Q, by which Q is caused to appear lighter, then the terminal portions of the Q-segments of the fibrils should become lighter first and the middle portions only subsequently. On the contrary, the portion nearest the point of alleged absorption, that is, the level between J and Q remains dense and deep-staining while the farthest removed level, namely, the midportion of Q, becomes light first.

No attempt will here be made to construct a modified theory of muscle contraction in accord with this important morphologic datum, but the point may be emphasized that any final theory of contraction must be able consistently to include this fact. A reversal of striations, in so far as the deep-staining constituent of the substance of the Q-disc of a muscle fiber in repose is concerned, seems demonstrated by the alterations in the chemical constituents, as indicated by alterations in staining reactions, of the several major stripes, including the obliteration

tion of the N-stripe of extended muscle. Holmgren⁷ claims that the contraction bands result from the aggregation of the J-sarcosomes about the telophragmata (p. 610), a position supported also by Retzius¹⁵ and by Heidenhain.⁵ Such claim is conclusively contravened by the fact that typical contraction bands appear both in fibers which normally lack these sarcosomes (fig. 8) and those in which they have been destroyed by alcohol (fig. 13).

This leads to the questions regarding the constitution and the significance of the accessory disc or N-stripe. This stripe has been described in the muscles of many insects. M. Heidenhain illustrates it even in the voluntary striped muscle of man (posterior crico-arytenoid; Plasma und Zelle, fig. 358, p. 622). In the leg muscle of the sea-spider this disc occurs in the form of a relatively pale band about midway between Z and Q, as previously described.⁹ In the leg muscle of the mantis it appears with exceptional clearness (figs. 2 and 3). It could not be detected in the wing muscles. The chief point of discussion concerns the location of its constituent elements, that is, whether the granules whose lateral juxtaposition results in this stripe are inter- or intrafibrillar (sarcostylic). Retzius¹⁵ and Heidenhain⁵ claim that the N-disc is composed of interfibrillar J-sarcosomes. Rollet¹⁶ interprets it as an intrafibrillar anisotropic constituent. That it does not consist of sarcosomes in the mantis muscle is demonstrated by the fact that it is present in the leg muscle of mantis after fixation in alcohol, which technic destroys sarcosomes, quite as definitely and distinctly as in muscle fixed in formalin, by which technic the sarcosomes are fully preserved. Moreover, in formalin-fixed wing muscle the smaller spherical intersarcostylic sarcosomes are segregated at certain phases of contraction close to the telophragma in the J-disc, but do not closely resemble the N-disc of the leg muscle.

In the leg muscle it can be clearly seen that the N-disc consists of intrafibrillar elements (figs. 2 and 3). The interfibrillar so-called J-granules of the wing muscle do not produce a genuine N-disc. It is, however, easy to see how confusion has arisen regarding the constitution of this N-disc. During contraction

the N-discs fuse with the deep-staining Q-substance in its passage to the telophragmata, and so contribute to the formation of the contraction bands of Rollet. Regular aggregation of J-sarcosomes may simulate N-discs, but genuine intrafibrillar N-discs and contraction bands are essentially independent of the interfibrillar sarcosomes. The data are not yet sufficient to permit of definite conclusions regarding the significance of the N-disc in relation to contraction, but that the facts of its occurrence and movement must be included in any complete theory of muscle contraction is obvious.

The matter of prime interest in the wing muscle of mantis concerns the sarcosomes. We should like to know their complete history, including origin, function, and fate, as well as structure and position. The study of their origin will be reserved for a separate paper. Their structure and their alterations in form and position at various phases of contraction have already been described. Holmgren⁷ has described similar elements in the wing muscle of *Libellula* and in the bumblebee and in the heart muscle of the crayfish. He figures and describes delicate connecting threads between successive sarcosomes. I have nowhere been able to detect such connecting fibrils in the mantis sections. Occasionally an underlying or overlying myofibril simulates a connecting thread, but the ease with which the granules separate both in the freshly dissected fibers and in the looser fibers of the sections, argues against the occurrence of such connecting fibrils in this tissue.

Holmgren classifies the sarcosomes of Retzius as J- and Q-granules. This raises one of the most difficult questions connected with this study. Do only sarcosomes of a certain specific type occupy the J-disc and only sarcosomes of a different type occupy the Q-disc? In an attempt to solve this problem areas like the one illustrated in figure 16 were carefully studied. Here the telophragmata are ruptured, the myofibrils widely spaced, and the sarcosomes lie freely within the interfibrillar areas. It is assumed that the sarcosomes maintained their normal mutual relationships during these modifications of the fiber. If this is an accurate assumption, it follows from the illustration that

there is no definitely regular succession of the several types of granules throughout as many as six successive sarcomeres. Large and small sarcosomes appear to be distributed indiscriminately. Moreover, in the intact fibers during repose (figs. 17 and 18) the several forms of granules are mingled in an area about midway between successive telophragmata, that is, in the region of the Q-disc. This is true also of their distribution in the contracted fiber (fig. 20). But during midphase of contraction (figs. 11 and 19), when Q becomes widened out, the two extreme size variations of sarcosomes are apparently segregated in different regions; the smaller, generally deep-staining, spherical granules are aggregated close about the telophragmata, while the larger, oval, generally lighter-staining granules are scattered in the newly formed H-disc of the dividing Q-substance. This segregation is the crux of the problem, and the explanation of this separation would seem to promise the clue regarding the genetic relationship of the several size variations of sarcosomes.

Reactions of the sarcosomes to alcohol and formalin and their staining reaction to iron-hematoxylin suggest that the sarcosomes of the several sizes are chemically similar, and that the larger are developed by growth from the smaller. The smaller, however, stain somewhat more intensely, and at the same time are more susceptible to the destructive action of water and glycerin in the fresh condition. The fact that the smaller sarcosomes move toward the telophragmata during contraction, considered in relation to the fact that during these stages there occurs a movement of intrafibrillar substance in the same direction, might seem to indicate that the smaller sarcosomes are actually intrafibrillar. But the direct histologic evidence clearly shows that these granules are actually interfibrillar. The explanation that seems most plausible, in view of all the available evidence, with regard to the segregation of the J- and Q-sarcosomes during contraction, is one expressed in terms of place of origin and the operation of mechanical factors. Assuming on the basis of admittedly meager histologic data (the mingling of the two types of sarcosomes in the Q-disc during certain functional phases) that the larger oval sarcosomes are later growth stages of the

smaller spherical sarcosomes, it may be that the newly formed young sarcosomes appear first in the outlying regions of the mass of Q-granules—that is, closest to the telophragma along which membrane, due to its connection with the sarcolemma, the elements from which the granules are formed presumably enter the fiber—and in consequence are forced against the telophragmata through the approximation of these membranes during contraction and the crowding of the larger oval Q-sarcosomes in the region midway between successive telophragmata. This is in accord with Holmgren's⁷ demonstration that the telophragmata are in close spatial relationship also with the terminals of the tracheae, through which oxygen is supplied to the muscle tissue, and with his interpretation of the Z-membrane as a path for the entrance and exit of products of metabolism. Holmgren,⁷ however, regards the J-granules as 'secondary degenerative fragmentation products' (p. 620), presumably resulting from the disintegration of the Q-sarcosomes whose function he conceives to be related to the supplying of the energy needs of the active muscle. In my opinion, the evidence supports better the interpretation which regards the J-sarcosomes as the precursors of the Q-sarcosomes than as products of their disintegration.

Bullard² has shown that lipid granules constitute a normal sarcoplasmic element of mammalian, including human, heart muscle. The question arises whether the sarcosomes of insect wing-muscle are the homologues of the lipid granules of mammalian heart muscle. There are a number of correspondences. A quotation from Bullard's paper³ will indicate the close similarity: 'Fat droplets of normal cardiac muscle are arranged in longitudinal and transverse rows in the sarcoplasm between the myofibrils or muscle columns. Large droplets are in the Q-band, smaller droplets in the J-band' (p. 28). Holmgren⁷ concludes for an identity both in the case of the heart of the crayfish and of mammals (p. 610). Retzius¹⁵ has described J-granules also in skeletal muscle of certain arthropods and even of the rabbit. In the latter material they are described as exclusively of the small spherical type. The fact that where only one type of granule is present it is of the smaller spherical type

speaks in favor of the above interpretation of the growth relationship between the smaller spherical and the large oval sarcosomes. The further fact that in these instances, as in certain skeletal muscles, the exclusive smaller sarcosomes are nearer the telophragma is in accord with our interpretation of their origin at this level. Heidenhain⁵ regards the sarcosomes as 'vegetative organelle,' places them in the category with secretory and pigment granules, and interprets them as reservoirs of the carbohydrates which are necessary for muscle function (p. 638).

Before discussing further the nature and function of the sarcosomes, we may consider an aspect of their microchemical reactions especially emphasized by Holmgren.⁷ Certain Q-granules stain more intensely peripherally where they abut upon the adjacent lamellae; the portion of the myofibril in contact with the sarcosome stains in a similar intenser manner. Holmgren interprets this phenomenon to mean that a substance passes from the Q-granules to the Q-segments of the myofibril. This seems plausible, but the possibility of an artifact may not be ignored. But Holmgren claims further that this condition corresponds to the extension phase; and that a very different specific condition, where the Q-granules shade laterally into wide deep-staining branches or wing-like processes, corresponds to the contraction phase and indicates an imbibition of the deep-staining substance from the more peripheral nucleated portion of the fiber. In the wing muscle of the mantis I can detect no sarcosomes with long wing-like processes such as Holmgren describes for the wing muscles of certain neuroptera. The only precise correspondence between sarcosome morphology and functional phase of the fiber is that represented by the change in the direction of the long axis of the oval granules between extension and contraction. However, accepting tentatively Holmgren's interpretation of his observations, we might conclude, on the basis of similar evidence, that the deep-staining substance which passes from the sarcosomes to the myofibrils is identical with that which passes from the Q-disc to the telophragma during contraction and so contributes to the formation of the contraction band, whence it is eliminated via the telophragma. Assuming

that the sarcosomes consist of a combination of carbohydrate and lipid elements, the deep-staining substance passing through the fiber to the Z-membrane might be thought to be in part the lactic acid resulting from the oxidation of the carbohydrate element of the sarcosome. The 'reversal' of the contraction band would, according to this interpretation, mean simply the elimination of the lactic acid at the telophragma and its reformation through oxidation of carbohydrates in the Q-disc. But this explanation, taken as a whole, seems too naïve to correspond faithfully to the series of undoubtedly very complex chemical reactions. Moreover, it ignores the fact that the same alterations occur during contraction in the stripes of muscle fibers which lack typical sarcosomes; and it is unable to adjust satisfactorily the discrepancy between the rapidity of muscle contraction and the time obviously demanded for the projection and retraction of long wing-like processes by a relatively rigid sarcosome. There is little doubt that the sarcosomes are related to the metabolic needs underlying the production of energy in active wing muscle, but the histologic picture probably cannot reveal all of the subtle physical and chemical changes involved in this activity.

In an attempt to arrive at conclusions regarding the function of the sarcosomes on the basis of their morphologic and microchemical alterations, the fact must be kept in mind that all striped muscle fibers have essentially an identical fundamental structure at the several functional phases whether the interfibrillar sarcoplasm contains definite sarcosomes or not. For the present, however, it cannot be definitely stated whether all striped muscle does or does not contain granular homologues, possibly much smaller and more susceptible to the destructive action of ordinary histologic technics, of the specific sarcosomes of insect wing muscle. The so-called sarcosomes of heart muscle may represent intermediate stages between the larger more resistant sarcosomes of insect wing muscle and their possibly more elusive homologues of skeletal muscle.

Without discussing at this point Bullard's implied identification of sarcosomes with mitochondria, we may consider his

suggestion that 'the anisotropic property of segment Q is dependent upon the presence of the phospholipines of the true interstitial granules' (p. 27).³ This would mean that the anisotropic condition of Q results from the presence of interfibrillar elements, instead of from intrafibrillar granules, as commonly assumed. I have carefully studied the wing muscle of mantis with the micropolariscope in an effort to determine the refractive properties of the sarcosomes. The fiber as a whole is distinctly anisotropic. But there seems to be no sharp segregation of the anisotropic substance in the Q-discs. It can, however, be easily determined that the Q-sarcosomes are not anisotropic. This demonstrates only that the anisotropic substance is extra-sarcosomic; but since the compact skeletal muscle fibers of mammals, in which relatively little besides myofibrillae is present, also are anisotropic as a whole, the conclusion seems to follow that the anisotropic materials are exclusively intrafibrillar.

In mammalian heart muscle Bullard³ has described two distinct types of 'interstitial granules' of Koelliker: fat droplets, and 'true interstitial granules' or mitochondria. The former he believes consist of neutral fat, and he interprets them as 'reserve foodstuff.' The fat droplets are said to correspond to the 'liposomes' of Bell;¹ the mitochondria to the J- and Q-granules of Holmgren and the sarcosomes of Retzius. Bullard, accordingly, would seem to interpret the sarcosomes of insect wing muscle as mitochondria. The accuracy of such interpretation is contravened by the fact that filar and bacillary mitochondria, such as are typical for differentiated somatic cells generally and for developing striped muscle fibers of vertebrates, are present in addition to sarcosomes (fig. 30). That sarcosomes and mitochondria have in part a similar chemical constitution is demonstrated by their similar microchemical reactions. Thulin²¹ also has called attention to the fact that the sarcosomes of *Hydrophilus* react in typical fashion to Benda's mitochondrial technic. Such similarity of reaction is probably due to the presence of a predominant lipoid constituent. The evidence seems rather to favor interpreting the sarcosomes of insect wings muscle as analogous to the fat droplets of mammalian heart

muscle, their function being related to the supply of nutrient materials for the muscle fiber.

This leads to the question of the relationship between mitochondria and fat droplets (and sarcosomes). The relation of the mitochondria to the sarcosomes in insect wing muscle will be the subject of a separate paper. But here it may be pointed out that Bullard regards his evidence as opposed to the view that true interstitial granules (mitochondria) metamorphose into fat droplets. On the contrary, however, Schreiner¹⁹ has very clearly shown that in the subcutaneous tissue of the hag-fish (*Myxine*) embryo, fat globules of developing fat cells do arise from mitochondria. The chromatic nucleolus here expels granules ('chromidia') which pass into the cytoplasm, where they become transformed into rodlets (mitochondria); these segment into secondary granules (liposomes) which liquefy to form fat vesicles, the latter subsequently coalescing to form the fat globule of a definitive adipose cell. Russo's¹⁷ experiments with rabbits are also of much interest in this connection. By the administration of lecithin (a typical phospholipine) he claims to have increased the mitochondrial content of the oöplasm, and coincidentally the size of the eggs and the quantity of their deutoplasmic constituents; and he records histologic data in favor of the deutoplasmic transformation of the mitochondria. That the injection of a phospholipine (lecithin) should increase the amount of mitochondria (largely phospholipines) is very significant from the view-point of mitochondrial function and their relation to nutrient fat.

These important observations of Schreiner and Russo, however, seem sharply contradicted by the results of the recent investigations of Lewis.¹⁴ In cultures of young chick embryos, grown in Locke's solution to which yolk stained with Sudan III had been added, Lewis finds no evidence indicating any association between the mitochondria and the accumulating fat droplets in the living cell. The conflicting, very positive conclusions especially of Schreiner and Lewis cannot at present be harmonized. The genetic relationship of mitochondria to liposomes (fat droplets) and to the sarcosomes of insect wing muscle still remains an open question.

SUMMARY

1. The salient differential features between the leg and wing muscles of mantis pertain to a conspicuous N-disc in the leg muscle fiber and sarcosomes in the wing muscle fiber.

2. The sarcostyles are of lamellar form peripherally and cylindrical form centrally. Both types contain constituent myofibrils. The sarcosomes are distributed in the intersarcostylic sarcoplasm. The myofibrils are directly continuous with the tendon fibrils.

3. The N-disc is formed by the lateral juxtaposition of intrafibrillar constituents. It fuses with a substance which moves from the Q-discs to the telophragma in the formation of a contraction band.

4. A contraction band (disc) includes the deep-staining substance of opposite halves of successive Q-discs, the two intervening N-discs and the inclosed bisecting telophragma. Both N-discs and contraction discs are independent of the sarcosomes.

5. The telophragma presents an effective barrier against the movement (passive) of the sarcosomes. No such barrier exists in the region commonly occupied by the mesophragma. Either a mesophragma does not occur in the wing muscles or it lacks an intersarcostylic component, that is, it may be fenestrated.

6. During certain functional stages, especially the midphase of contraction, the sarcosomes are more or less sharply segregated into J and Q groups.

7. The sarcosomes have an initial spherical shape. This may become modified by pressure into oval or irregular forms. They have a semifluid consistency and are enveloped by a more condensed peripheral 'membrane.' They have in large part a lipoid chemical constitution, as indicated by their reactions to fat solvents and stains. The smaller J-sarcosomes are apparently the precursors of the older larger Q-sarcosomes. The aggregation of the J-sarcosomes nearer the telophragma is to be interpreted in terms of their origin from materials transported by the telophragma from the interfiber tissue spaces. The final phase of the sarcosomes as hollow vesicles, frequently

collapsed, indicates a transient nature and suggests a nutritive significance. The persistence of a granular débris after treatment with alcohol indicates that they contain an albuminoid or carbohydrate constituent in addition to the major lipid element.

8. The sarcosomes present chemical and staining reactions similar to mitochondria. Their cytoplasmic constitution is obviously closely similar. But sarcosomes are more closely analogous to fat globules than to mitochondria. The genetic relation between mitochondria, sarcosomes, and fat globules remains undetermined.

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EXPLANATION OF FIGURES

The drawings were made with the aid of an Abbe camera lucida. Unless otherwise specified, the tissues illustrated are from adult individuals, fixed in strong Flemming's fluid, and stained with iron-hematoxylin; and the magnification is 1500 diameters.

PLATE 1

EXPLANATION OF FIGURES

Leg muscle

- 1 Longitudinal section of portion of fiber, in the extended or resting condition. *z*, telophragma. Formalin fixation.
- 2 Similar fiber, more deeply stained, showing the accessory disc, or N-line. Formalin fixation.
- 3 Similar fiber in early phase of contraction, showing the additional H-disc and the E-disc. Alcohol fixation.
- 4 Later phase of contraction. The accessory discs (N) have fused with the separating halves of *Q. Nu.*, nucleus. Alcohol fixation.
- 5 Slightly later stage, showing the beginning of the formation of the contraction band (C.B). Formalin fixation.
- 6 Similar fiber, less deeply stained, showing the festooned sarcolemma (S) and an underlying nucleus. Formalin fixation.
- 7 Later stage in contraction. Alcohol fixation.
- 8 Fully contracted fiber. *C. B.*, contraction band. Formalin fixation.

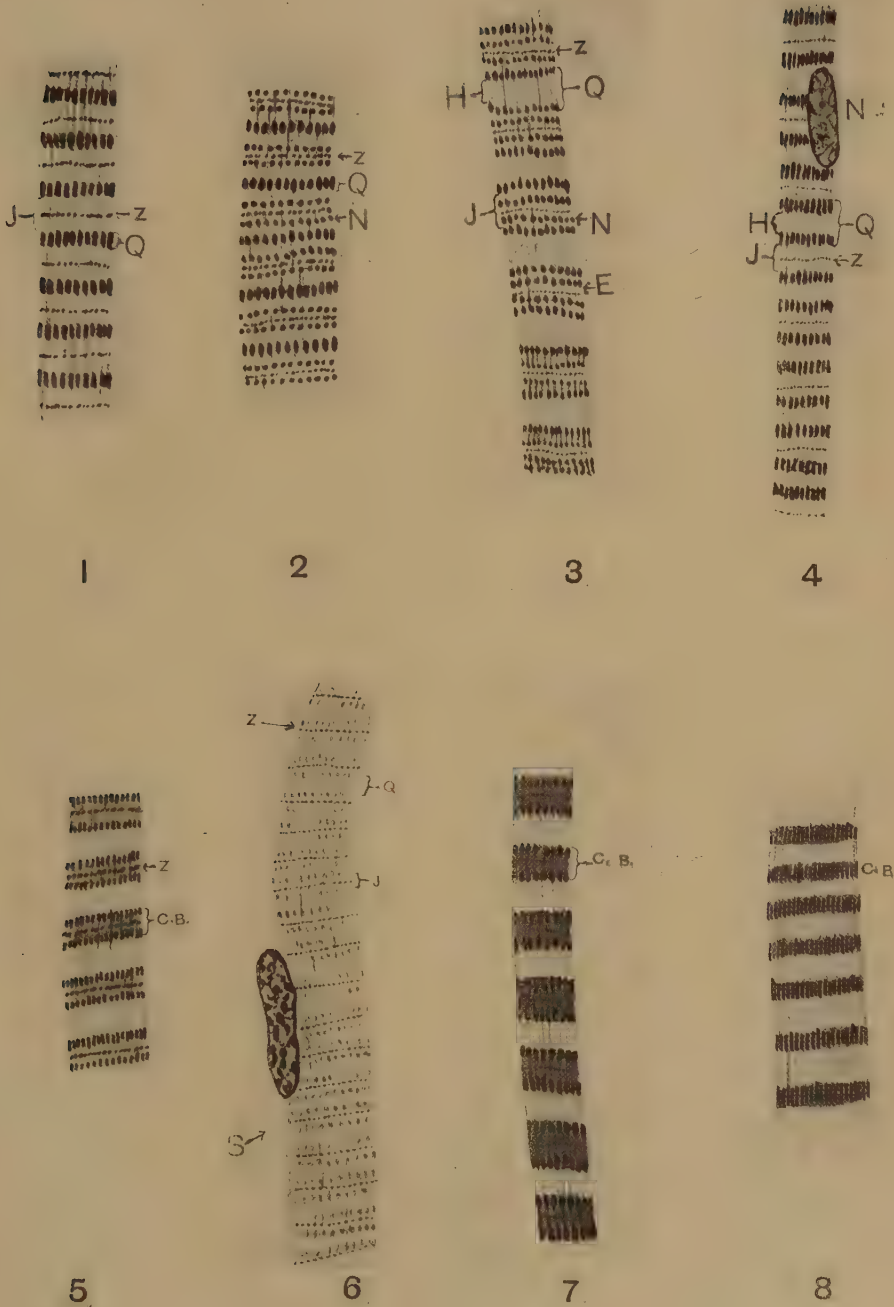


PLATE 2

EXPLANATION OF FIGURES

Wing muscle

9 Longitudinal section of portion of fiber in extended or relaxed condition, lightly stained. The sarcosomes are not shown. Formalin fixation.

10 Similar fiber in early phase of contraction. Formalin fixation.

11 Later stage in contraction. This fiber, which is slightly stretched, shows also the so-called J- and Q-granules or sarcosomes. Formalin fixation.

12 Beginning formation of contraction band (C. B.) The sarcolemma (S) is festooned between the telophoragmata (Z). Flemming fixation.

13 Fully contracted fiber, showing contraction bands (C. B.) and the festooned sarcolemma (S). Flemming fixation.

14 Portion of fiber in which the sarcostyles have become widely separated and the telophoragmata (Z) ruptured, showing the several types of sarcosomes: oval and spherical; larger and smaller; deeply and lightly staining; solid and vesicular.

15 Portion of fiber in which the telophoragmata-sarcostyles connection remains intact. The sarcosomes appear as hollow spheroidal globules, the sarcostyles curving around them between successive telophoragmata. This fiber is in the contracted condition.

16 Four adjacent interfibrillar spaces, showing the irregular distribution of the several types of sarcosomes, including modified cup-shaped forms.

17 and 18 Lightly stained fibers in the extended condition, showing the more regular type of sarcosome distributed midway between successive telophoragmata.

19 Similar fiber in early stage of contraction, showing the distribution of the J- and Q-sarcosomes. (Compare with figure 11.)

20 Lightly stained fiber in the contracted condition. The oval sarcosomes, with their long axis previously parallel to the long axis of the fiber, have changed to an oval condition in which the long axis is now at right angles to the long axis of the fiber, as if modified by a pressure resulting from the closer apposition of successive telophoragmata.

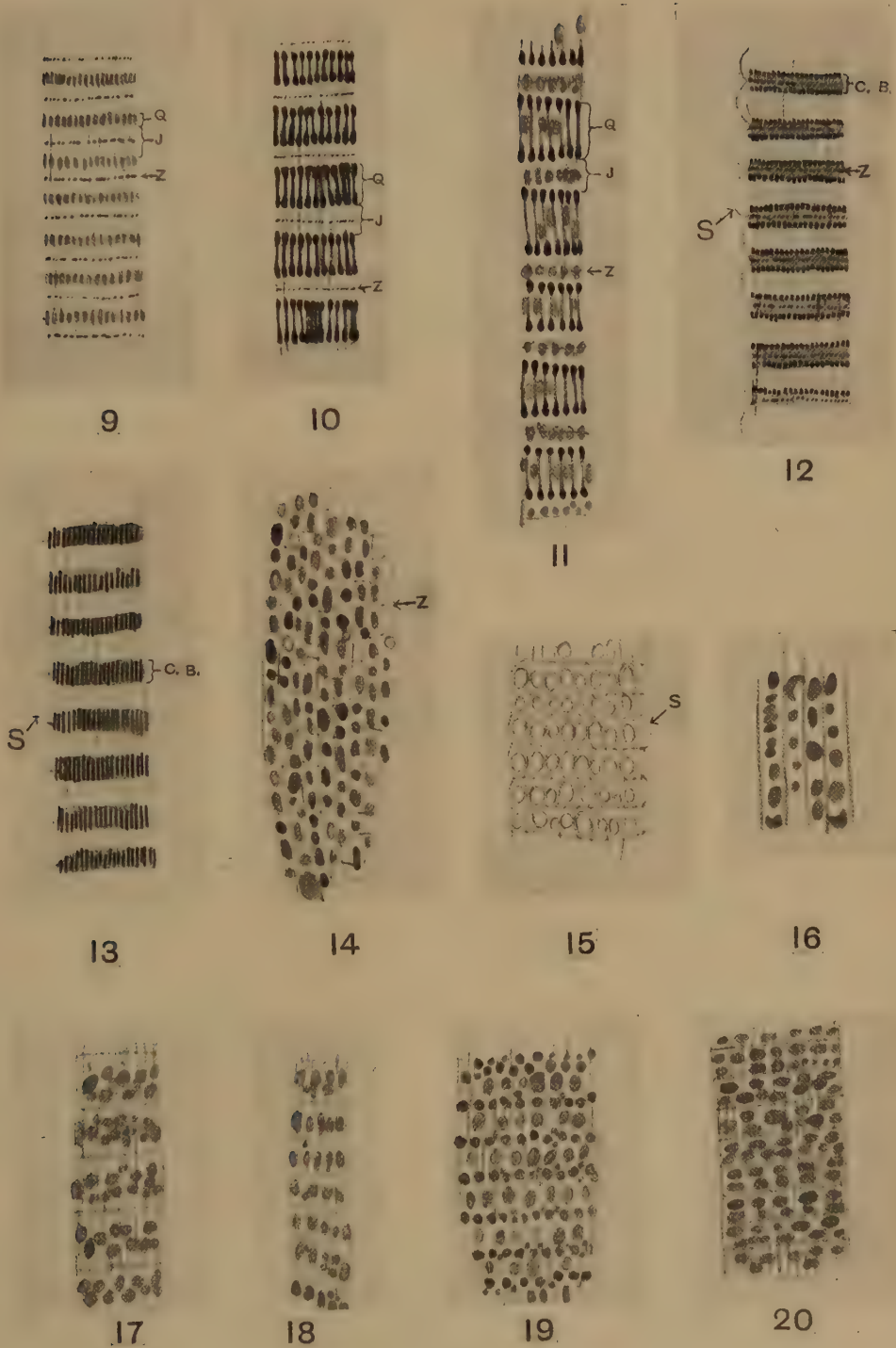


PLATE 3

EXPLANATION OF FIGURES

21 Longitudinal section of portions of two adjacent fibers. The fibers are only lightly stained. The nuclei (*Nu*) are very numerous and peripherally placed. *S*, sarcolemma; *Z*, telophragma. Drawing by Mr. Massie Page. $\times 600$.

22 Transverse section of four adjacent fibers. The peripheral sarcostyles are of the lamellar type, the central of the cylindric type. *Ec*, ectoderm; *S*, sarcolemma. Drawing by Mr. Massie Page. $\times 600$.

23 Transverse section of a fiber, showing one peripheral and one subcentral nucleus, also a connective-tissue nucleus. The peripheral sarcostyles (*L*) are chiefly lamellar, the more central cylindric. Many of the peripheral lamellae are in process of longitudinal radial division. Scattered among the sarcostyles, through the central, peripheral, and perinuclear sarcoplasm, are many sarcosomes (*Ss.*). $\times 1000$.

24 Transverse section of a smaller looser fiber, in which the nucleus is centrally placed, the sarcostyles are almost exclusively of the cylindric type (only the peripheral border containing narrow lamellar sarcostyles), and the sarcosomes are more abundant and conspicuous. $\times 1000$.

25 Longitudinal section of small area of muscle connected with the ovipositor, showing nuclear multiplication both by the mitotic and the amitotic (*X*) modes. These fibers show faint cross striations, the telophragmata. Aceto-sublimite fixation.

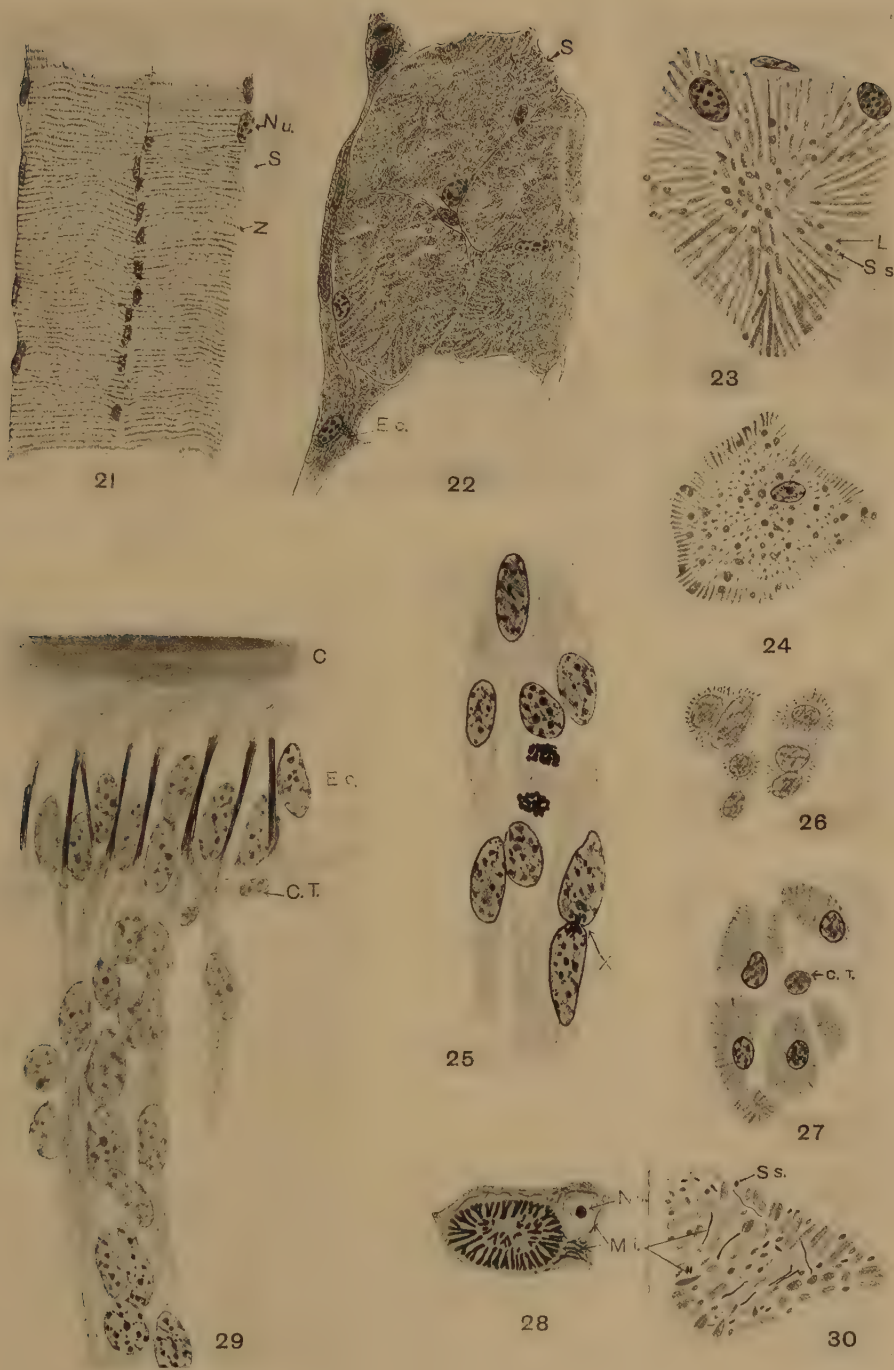
26 Transverse section of similar group of young muscle fibers, showing the central location of the nuclei and the narrow lamellar form of the initial sarcostyles.

27 Transverse section of a group of older muscle fibers. The nuclei are beginning to move peripherally, and the sarcostyles are coarser lamellae. *Ct.*, connective-tissue nucleus.

28 Transverse section of young muscle fiber of newly hatched rainbow trout. The sarcostyles include peripheral lamellae and central cylinders, both in process of longitudinal division. *Nu.*, nucleus; *Mi.*, mitochondria. The similarity between young vertebrate voluntary striped muscle and adult insect muscle (fig. 23) is striking. Meves' technic. $\times 1000$.

29 Longitudinal section of group of young muscle fibers connected with the ovipositor. The nuclei increase in number towards the point where the muscle fibrils pass between the hypodermal cells (*Ec*) to become inserted into the chitin (*C*). There is here strict continuity between muscle fibrils and tendon fibrils. *C.T.*, basement membrane. $\times 1000$.

20 Transverse section of wing-muscle fiber, showing filar and bacillary mitochondria (*Mi.*) among the sarcosomes (*Ss.*) and myofibrils. $\times 1000$.



Resumido por el autor, Hubert Dana Goodale.

Las células intersticiales en las gonadas de la gallina doméstica.

El autor ha encontrado abundantes células con el tamaño, forma y reacciones colorantes de las llamadas células intersticiales del ovario de la gallina, en el timo, en el testículo del macho bajo ciertas condiciones y en la sangre. Con menos frecuencia tales células se han observado en otros órganos. Las células intersticiales, por consiguiente, son probablemente, leucocitos eosinófilos.

Translation by José F. Nonidez
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INTERSTITIAL CELLS IN THE GONADS OF DOMESTIC FOWL

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FOUR FIGURES

The literature on interstitial cells of the reproductive organs has been adequately reviewed by Boring and Pearl ('17). In the same paper they have reexamined the question and reach the conclusion that the 'true' interstitial cells, while always present in the ovary and usually, if not always, absent from the adult testis, are not causally concerned with the control of the secondary sexual characters. This conclusion is substantiated by later papers, viz., Boring and Pearl ('18) and Pearl and Boring ('18).

Light is thrown on the nature of these cells by certain observations made in this laboratory, in the course of which several differential stains, including those employed by Boring and Pearl, were used. They indicate that the granule containing cells, to which the term 'interstitial' is limited by Boring and Pearl, are eosinophils. If this view is substantiated by further studies, it will explain both the irregularities in their distribution in the ovary and the discrepancies in the accounts of various authors as to their occurrence or non-occurrence in the testis.

The evidence for the view that the granular interstitial cells are leucocytes is as follows: Cells of the same size, shape, having granules of the same size, and taking the same stains as do the granular cells of the chicken ovary are to be found in other organs, abundantly in some, rare in others. They may occasionally be observed interspersed among the erythrocytes in blood-vessels.

The most favorable material encountered, aside from the ovary, is the active thymus of two molting drakes. Here these cells

are located in great abundance along the trabeculae (fig. 1).¹ A few are found distributed among the lymphoid cells, but in any case they are sharply differentiated from the latter. In other instances, these cells are absent or few in number. Eosinophils are mentioned as normal constituents of the thymus in various text-books of histology.

In the tunica albuginea of the testis of the same individual a few of these cells were observed, though absent from the tubules.

In figure 2 are shown the same sort of cells in the connective-tissue covering of several tubules of the testis of an old hen-feathered Silver Spangled Hamburg cock, which are undergoing cystic degeneration. They are absent from normal portions of the same testis.

Figure 3 illustrates the same sort of cells in the epididymis of a drake's testis, both in the connective tissue and in the blood stream.

Figure 4 shows the granular cells near the surface of a portion of a hen's ovary.

Thyroid, pineal, and pituitary glands have been stained and search made for cells with the same characters. They have been found, though they are not common. Doubtless they could be found in other organs, but no search has been made.

The relative abundance of granular interstitial cells in the ovary can easily be understood if these cells are really leucocytes, since the ovary is the active seat of both progressive and regressive processes. Their presence in the degenerating tubules of the Hamburg cock's testis, but not in the normal portions, can be explained on the same basis as well as the observed irregularities in their occurrence in the thymus. Such gatherings of leucocytes are a well-known phase of their behavior. While these granular cells are usually very abundant in the ovary, one specimen of ovary from a nine-year-old hen with well-developed spurs is noticeable for an almost complete absence of such cells. This may be due to the fact that the greater part of the tissue consists of luteal cells.

¹All figures, though outlined with a camera, have been somewhat schematized. The cells are relatively too large.



Fig. 1 Thymus of molting drake. *I*, interstitial cells; *BV*, blood vessel; *P*, parenchyma. $\times 120$.

Fig. 2 Cystic spermatic tubules of hen-feathered Silver Spangled Hamburg cock. *I*, interstitial cells; *BV*, blood vessel; *Sz*, spermatozoa; *Sm*, spermatogonia; *C*, connective tissue. $\times 120$.

Fig. 3 Diagrammatic representation of epididymis, *Ep*, and adjoining spermatic tubules, *St*, of a drake. *I*, interstitial cells. $\times 80$.

Fig. 4 Portion of ovary near the surface. *St*, stroma; *I*, interstitial cells. $\times 452$

In addition to cells that appear to be ordinary connective-tissue cells, the stroma of the chicken's ovary contains a cell type that stains poorly and which have been called clear cells by some authors, but which Pearl and Boring designate as luteal cells. Although no granules have been demonstrated, the possibility must be recognized that these cells may be true interstitial cells, at least from the physiological standpoint, i.e., they may furnish an internal secretion such as is demanded of the ovary.² The fact that they are not demonstrably granular does not militate against this view, since special treatment may be required to demonstrate granules (for example, Bensley, '16.) or the secretion may never be stored in granular form.

There are two further observations that bear on the relation between the gonad and the secondary sexual characters. The first is that the various sorts of cells observed in the normal ovary are found in ovarian tissue transplanted into castrated males with resulting feminization of the subject. The second is the presence of yellow pigment, quite like that found in the ovary, in the epididymis of a drake. It is possible that this pigment may have a causal connection with the development of the summer plumage. These matters will be dealt with later.

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²After the manuscript of this paper was completed, my attention was drawn to the presence of luteal cells in the testes of hen-feathered males by Professor Morgan. An examination of the testes of a young Brown Leghorn male (as adult Brown Leghorns are cock-feathered) showed similar cells, as did pieces of testes taken from a transplant beneath the skin of a castrated Brown Leghorn male, which at maturity was cock-feathered. A further study of such cells and their distribution is being made.

Resumido por la autora, Ruth Rand Atterbury.

La bolsa y tonsila faringeas; nota sobre sus relaciones en el embrión de vaca.

El presente estudio es preliminar de una investigación sobre el origen de la tonsila faríngea. Schwabach (1888) y Huber (1912) han demostrado que dicha estructura se desarrolla en el hombre en el punto en que está colocada la bolsa faríngea. Schwabach interpreta la bolsa faríngea como el primer esbozo de la tonsila y es equivalente al seno tonsilar de la tonsila palatina. Según Huber, la bolsa faríngea del hombre nace por la persistencia, en un cierto sitio, de la conexión primitiva entre el notocordio y el epitelio faríngeo. Una investigación verificada por la autora sobre el embrión del cerdo (Rand 1917) ha demostrado que los contactos notocordales notados por los autores citados son secundarios y no están relacionados, en modo alguno, con el origen de la bolsa faríngea, sino que los divertículos faríngeos se desarrollan en íntima asociación con la fascia faringo-basilar. Las observaciones llevadas a cabo sobre el embrión de vaca demuestran: 1) Que no hay nada que corresponda a los contactos notocordales primarios descritos en el embrión humano ni con los contactos secundarios observados en el cerdo. 2) Que la fascia faringo-basilar, en general, no viene a ponerse en relación directa con el epitelio faríngeo como se ha descrito en el cerdo. 3) La tonsila faríngea se desarrolla con completa independencia de los divertículos faríngeos. De aquí que la bolsa faríngea no pueda considerarse como esencial, o directamente relacionada, con la aparición de la tonsila faríngea. Esta última, en la vaca, se desarrolla en un área que coincide generalmente con las fibras terminales de la fascia faringo-basilar.

Translation by José F. Nonidez
Columbia University

BURSA AND TONSILLA PHARYNGEA; A NOTE ON THE RELATIONS IN THE EMBRYO CALF ¹

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EIGHT FIGURES

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The following study was undertaken as a preliminary step in an investigation and interpretation of the pharyngeal tonsil. As is well known, the pharyngeal tonsil of man arises in close anatomical association with a small blind pocket in the roof of the nasal pharynx, called the bursa pharyngea or recessus medius pharyngis. Figure 14 a of Huber ('12) clearly shows this association. The problem of the relation of this pocket to the pharyngeal tonsil has long occupied the attention of many investigators and has given rise to two fairly well-defined interpretations. According to one view, the bursa pharyngea is an integral part of the pharyngeal tonsil, of no significance as a structure apart from the tonsil. Thus Schwabach ('87), in recognition of the prior development of the bursa pharyngea, designates it as the first anlage of the pharyngeal tonsil. According to his observations, the lymphocytes appear first in the region of the bursa and here become most highly developed. Schwabach was interested in drawing an analogy between the bursa pharyngea of the pharyngeal tonsil and the sinus tonsillaris of the palatine

¹ The writer desires to express her deep appreciation of the very generous help of Dr. Kingsbury in this work.

tonsil, believing the two to be equivalent structures. According to the second interpretation, of which Killian ('88) was the first advocate, the bursa pharyngea is a structure sui generis, entirely distinct from and independent of the pharyngeal tonsil.

Since Killian, investigators have been concerned chiefly with the origin of the bursa pharyngea as a structure in itself, irrespective of any relation to the pharyngeal tonsil. The work has been done for the most part on human embryos. In general, the tendency has been to regard the bursa pharyngea as a mechanical structure arising in the growth of the pharyngeal region, primarily through the maintenance at a certain point of the primitive connection between the notochord and the pharyngeal epithelium. Huber ('12) makes a very clear statement of this view. Since notochordal contacts with the pharyngeal epithelium in pig embryo had been noted by observers (Mrs. Gage, '06, and Mead, '09), and since in the pig there is present a pharyngeal pocket which has been considered homologous to the bursa pharyngea of man, Rand ('17) extended the study of the origin of the bursa to include this form. As the result of her observations it was shown that, 1) those notochordal contacts observed in the pig embryo were but secondary and seemingly accidental, in no wise concerned in the origin of a pocket homologous to the bursa pharyngea of man; 2) in the pig embryo a series of epithelial pockets, the most caudal one of which corresponds in anatomical relations with the bursa pharyngea of man, arises in close association with the connective-tissue strands of what has been termed the fascia pharyngobasilaris. Hence the suggestion was made that it is not merely maintenance of notochordal contact, but equally the connective-tissue relation which is responsible for the epithelial outpocketing (bursa pharyngea) in man.

As is known, the cow is a form which possesses a well developed pharyngeal tonsil. But to our knowledge it has never been reported that the notochord maintains contact with the pharyngeal epithelium in the cow, nor that in the cow epithelial outpocketings develop corresponding to the bursa pharyngea of man or the series of pockets in the pig. It was thought

important to ascertain these points and to determine whether or not a bursa pharyngea in the calf embryo is in any way involved in the development of the pharyngeal tonsil. It may be stated at once that the results of the investigation in the calf embryo show that, 1) there is nothing to correspond either to the maintenance of primitive notochordal contacts as described for the human embryo or to those secondary contacts observed in the pig; 2) the roof of the nasal pharynx shows no development of a bursa pharyngea or epithelial outpocketings; 3) the pharyngeal tonsil appears as diffuse adenoidal tissue in the mucosa of the nasal pharynx quite independent of the presence of any pharyngeal pocket. Hence we cannot follow Schwabach in regarding the bursa pharyngea as in any way essential to, or directly correlated with the appearance of the pharyngeal tonsil.

MATERIAL AND METHODS

The study is based on an examination of the pharyngeal region of a series of calf embryos ranging in length from 3.5 to 445 mm. crown-breech measurement. For the younger stages the excellent embryos belonging to the Cornell collection were used. These embryos were fixed in picro-aceto-formol and were stained with hematoxylin and orange G. In addition to these, dissected regions including the basilar plate and the entire nasolaryngeal pharynx were especially prepared from a series of older embryos, ranging in length from 48 to 445 mm. These were fixed in picro-aceto-formol or formalin and stained in toto with Mayers hydrochloric acid carmine for purely morphological work. Lyon's blue proved very helpful as a counterstain in bringing out clearly the connective-tissue fibers. The following list gives the length in millimeters of those embryos which were especially studied: 3.5, 7, 9, 11, 11.5, 14, 14.5, 20, 21, 23, 23, 24, 25, 25, 27, 28, 30, 48, 58, 62, 70, 80, 80, 82, 82, 92, 110, 130, 140, 163, 190, 235, 245, 305, 445. With few exceptions, the embryos were cut in the sagittal plane. The figures consist of reconstructed drawings of the pharyngeal region in the midsagittal plane and were made with the aid of the projection microscope.

OBSERVATIONS AND DISCUSSION

Relation of notochord to pharyngeal epithelium

The relation of the notochord to the pharyngeal epithelium in the calf embryo differs from that described for both the human and the pig embryo. The youngest calf embryo at our disposal (3.5 mm. in length) showed the notochord completely separated off from the pharyngeal entoderm except at its extreme cephalic end, which is in contact with a slight invagination just posterior to Rathke's pocket. This is the region of Sessel's pocket, but in the calf, as in the human, the pocket is hardly to be detected. The relations are very similar to those figured by Tournoux ('12) for a 10-mm. calf embryo. In the pig embryo, on the other hand, Sessel's pocket is more highly developed and is clearly recognizable till the 15-mm. stage.² In

² From the fact that the human bursa pharyngea arises in close relation with notochordal contact and the fact that connections between the anterior end of the notochord and Sessel's pocket had been observed in chick, rabbit, and sheep embryos, R. Meyer ('10) was led to identify the bursa pharyngea in man with Sessel's pocket. This statement has been successfully refuted by Huber, who has shown that the region of Sessel's pocket in the human embryo is separated from the bursa pharyngea by nearly the length of the vault of the pharynx. Recently Radford ('13) has published a note on the condition found in the ferret embryo, in which he disputes this point with Huber. Radford figures Rathke's pocket in the ferret embryo, just back of which is a small Sessel's pocket. The apex of Sessel's pocket ends in a solid cord of cells, which, according to Radford, resemble notochordal cells, but which lack a notochordal sheath. Granting that this solid cord of cells is the remnant of a primitive notochordal union, the evidence is not in the least convincing that this pocket (Sessel's) is homologous to the bursa pharyngea of man, and that the bursa pharyngea of man is therefore identical with Sessel's pocket. It is generally conceded that the region of Sessel's pocket is just caudal to Rathke's pocket. Huber has shown conclusively that the human bursa pharyngea arises at a point which is separated from Rathke's pocket by nearly the length of the vault of the pharynx. Radford's criticism that Huber does not figure Sessel's pocket and the bursa pharyngea simultaneously in the human embryo is unjustified, because Sessel's pocket, although well developed in the embryos of many vertebrates, is generally absent or but poorly developed in the human embryo. Moreover, it can not be said that Radford's figure in any way resembles that of Mead for a 30-mm. pig embryo, in which the notochord at the end of its ventral flexure through the basilar plate twice comes in contact with the pharyngeal epithelium. Radford's points of contact are just posterior to Rathke's pocket; Mead's notochordal contacts are at a level corresponding to the middle of the basilar plate, separated from the

all the older calf embryos examined this notochordal contact with the region of Sessel's pocket is lost, the anterior end of the notochord lying free in the mesoderm posterior to Rathke's pocket. This condition is similar to that figured for the human embryo (Huber and Tourneux). It has been shown in the pig embryo that after the cephalic tip of the notochord has lost contact with Sessel's pocket, it exists free in the mesoderm for a time, and then regularly comes in contact with the posterior wall of Rathke's pocket. In the calf embryo, as has been figured for the human embryo, the anterior end of the notochord does not regularly come in contact with Rathke's pocket. This is additional evidence for regarding the notochordal contact with Rathke's pocket in the pig embryo as secondary and accidental, in no wise concerned with the origin and formation of the anterior lobe of the hypophysis, as recently maintained by M. M. Miller ('15).

In the calf embryo there was nothing to correspond either to those primary contacts which the notochord maintains with the pharyngeal epithelium described by Huber for the human embryo, or to the secondary contacts which the notochord acquires with the pharyngeal epithelium, as described by Rand for the pig embryo. Calf embryos 7 to 14 mm. in length show the notochord entirely separated off from the pharyngeal entoderm, passing forward over the pharyngeal roof in a straight, even course, ending cephalad in a slight ventral turn in the mesoderm immediately caudal to Rathke's pocket. Figure 1 of a 9.5-mm. calf embryo shows the typical condition at this stage. In later stages the notochord bears practically the same general relations to the basilar plate as that described for the pig embryo. Entering obliquely through the dorsal surface of the posterior end of the basilar plate, it passes through to the ventral surface in a long curve, thence diagonally dorsal, ending in a 'hook' near the sella turcica. In a large percentage of embryos examined,

region of the hypophyseal diverticulum, which at this stage has completely lost connection with the oral cavity by half the length of the basilar plate. It cannot be said that Radford's criticisms of Huber's point are in any way valid, and indeed he himself admits the slightness of his evidence.

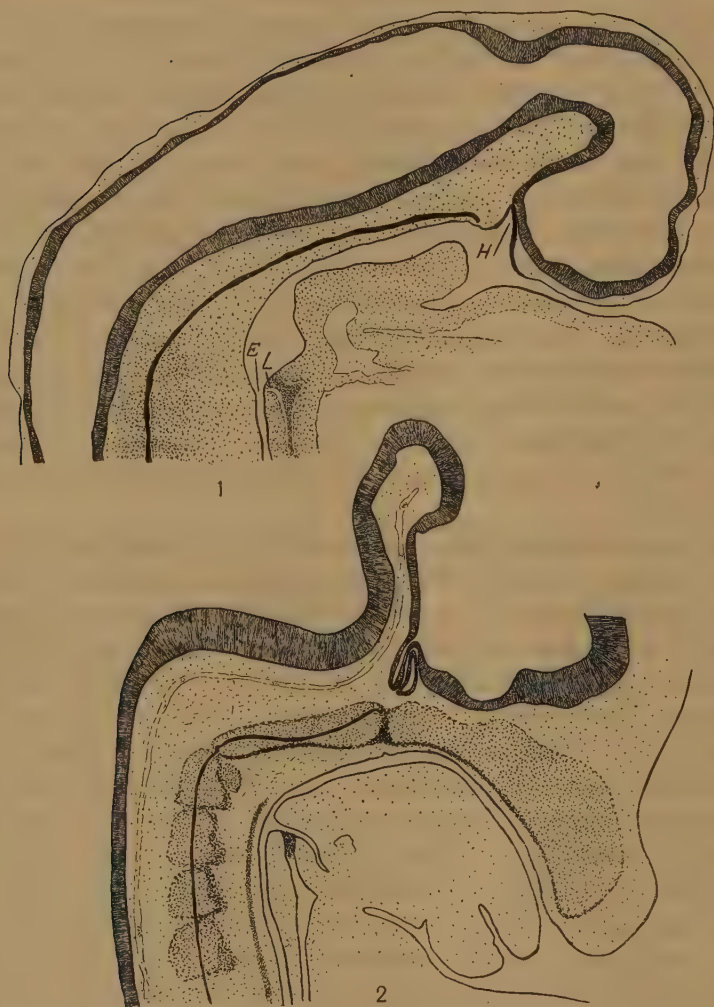


Fig. 1 Calf embryo, 9.5 mm. Reconstruction of the middle plane in the pharyngeal region. Notochord black. *H.*, hypophysis; *E.*, esophagus; *L.*, larynx. $\times 20$.

Fig. 2 Calf embryo, 23 mm. Reconstruction of the median sagittal plane, including the floor of the neural tube, basilar plate, etc., and the pharyngeal region. $\times 10$.

the anterior end of the notochord runs along the very edge of the sella turcica in a way similar to that figured by Tourneux for a 30-mm. calf embryo (fig. 2). In one embryo, 48 mm. in length, the notochord at the end of its ventral flexure through the basilar plate left the basilar plate, running for a short distance beneath it, to enter again and pass in the characteristic diagonal course toward the sella turcica. This condition is similar to that described by Tourneux for the embryos of the wolf, dog, cat, and man, but in the calf it is exceptional. In no case did the notochord leave the basilar plate to come in contact with the pharyngeal epithelium, as has been described for the embryos of man (Huber, Tourneux), the pig (Mead, Rand), and the horse (Tourneux).

Epithelial outpocketings and connective-tissue relations

There is nothing in the calf embryo to correspond to the temporary outpocketing described in pig embryos of 10 to 12 mm. length (Minot '11, and Rand, '17), which arises at the apex of the pharynx apparently as a result of growth tensions due to the flexion of the head at this time. Neither is there anything in the calf embryo to correspond to the large pharyngo-esophageal recess of the pig embryo, which arises in close relation with the developing pharyngeal musculature. The condensation of mesenchyme indicating the anlage of pharyngeal musculature is apparent over the roof of the laryngeal pharynx by the time the embryo attains a length of 15 to 20 mm. (fig. 2). This condensation of mesenchyme in the calf embryo, however, does not come so closely into relation with the pharyngeal epithelium as it does in the pig embryo.

In the preliminary study of the nasal pharynx of the pig embryo, Rand figures the pharyngeal region of a 54-mm. pig embryo which shows a series of three pockets, the most caudal one of which is most highly developed and bears the characteristic anatomical relations of the bursa pharyngea of man. These pockets in the pig, however, arise independently of notochordal contact, being closely associated in their origin with the fibers

of the fascia pharyngobasilaris. Special attention was paid therefore to the possible development of similar pockets in the calf embryo. It was found that epithelial outpocketings do not regularly develop. However, of the nineteen embryos examined over 48 mm. in length, three, measuring 110, 130, and 163 mm., respectively, proved exceptional cases, showing a shallow depression in the roof of the nasal pharynx. These depressions were situated just in front of the upper limits of

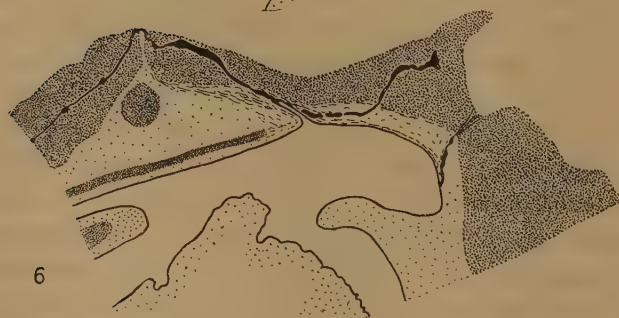
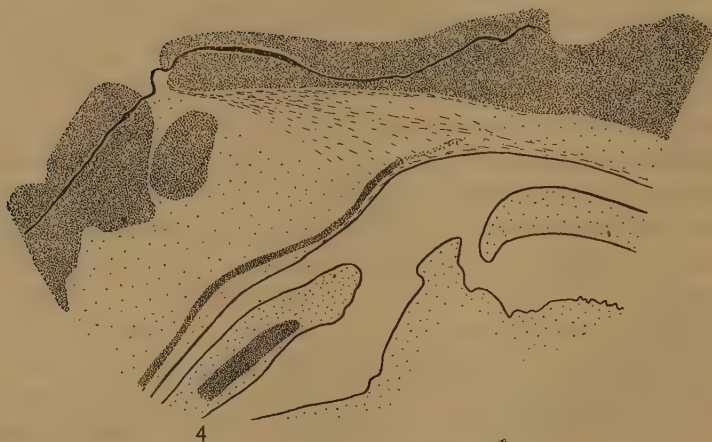


Fig. 3 Calf embryo, 110 mm. Reconstruction of the median sagittal plane of the pharyngeal region. $\times 10$.

the superior constrictor pharyngis at about the level of the middle of the basilar plate, corresponding therefore in anatomical position to the bursa pharyngea of man and the most caudal of the series of pockets in the pig. In the 110- and 130-mm. calf embryos the pockets were in definite relation with the fibers of fascia pharyngobasilaris (fig. 3). In the 163-mm. embryo the connective-tissue relations to the epithelial outpocketing were not so clear, due to the presence of lymphocytes in the mucosa of this region. In none of the calf embryos examined

did more than this single outpocketing develop. However, it was noted that anteriorly the fibers of the fascia often came into relation with pharyngeal epithelium in such a way as to give the appearance of slight 'pulls' accompanied by an epithelial and connective-tissue disturbance (fig. 3).

Since the appearance of these depressions or outpocketings was exceptional, a careful examination of the relation of the fascia to the pharyngeal epithelium was made in those calf embryos in which the pocket failed to develop. The first indication of the fascia is in the form of a condensation of mesenchyme close under the basilar plate, clearly discernible in embryos which have attained a length of 20 mm. (fig. 2). This condensation of mesenchyme does not become clearly differentiated as fibrous connective tissue until the embryo has become 40 to 50 mm. in length, when it is still in close relation with the basilar plate. In the mean time the pharyngeal musculature has continued to develop, so that in a 58-mm. embryo its anterior limit in the medial plane is somewhat anterior to the level of the end of the ventral flexure of the notochord as it courses through the basilar plate. In older calf embryos the posterior portion of the fascia may be seen extending toward the pharyngeal epithelium but the course of the fibers is interrupted by the anterior portion of the developing pharyngeal musculature. In this way the fibers of the fascia pharyngobasilaris of the calf embryo do not regularly come into so direct a relation with the pharyngeal epithelium as they do in the pig embryo. Anteriorly, beneath the sella turcica and the basisphenoid, the fibers run in thick bundles parallel with the pharyngeal epithelium, sometimes showing the slight 'pulls,' of which mention has been made above. A very characteristic picture is that shown in figure 4 of a 62-mm. calf embryo, in which the fibers of the fascia pharyngobasilaris may be seen in the preserved specimen, actually diverted from their course toward the pharyngeal epithelium by the anterior limits of the developing musculature. Often the fibers appear to extend down in among the muscle cells, thereby coming into a less direct relation with the pharyngeal mucosa, as in figures 3 and 4. The conclusion was there-



fore reached that in the calf embryo, the growth relations of the pharyngeal region are ordinarily of such a nature that the direct course of the fibers of the fascia pharyngobasilaris toward the pharyngeal epithelium is interrupted in its posterior portion by the developing superior constrictor pharyngis. In certain individuals, however, shiftings may occur so that the fibers do come into direct relation with the pharyngeal epithelium, at a point just anterior to the upper limits of the developing superior constrictor pharyngis. In such cases a pocket may develop corresponding in anatomical relations to the bursa pharyngea of man and the similar outpocketings in the pig.

Figure 4 of a 62-mm. calf embryo, figure 5 of a 50-mm. pig embryo, and figure 6 of a 46-mm. human embryo are arranged together, in order that a direct comparison may be made of the typical relations of the pharyngeal region in each of the three forms, respectively. In the calf embryo the fibers of the fascia pharyngobasilaris do not regularly come into direct relation with the pharyngeal epithelium, and in the calf a pharyngeal pocket does not ordinarily develop. In the pig embryo the fibers of the fascia regularly come into direct relation with the pharyngeal epithelium, and in the pig a series of two or more epithelial pockets regularly arises in close association with the fascia pharyngobasilaris. The figure of the human embryo shows the bursa pharyngea developing in close relation with the notochord, as has been described by Huber. Attention is called, however, to the rather thick sheath of connective tissue surrounding the notochord, which is also in close relation with the epithelium of the bursa. The condition is reproduced (in

Fig. 4 Calf embryo, 62 mm. Reconstruction of the median sagittal plane of the pharyngeal region. $\times 10$.

Fig. 5 Pig embryo of 50 mm. total length. Reconstruction of the median sagittal plane of the pharyngeal region, showing two small outpocketings of the pharyngeal epithelium related to strands of the 'fascia pharyngobasilaris. $\times 10$.

Fig. 6 Human embryo (no. 93Ha, Cornell Collection), 46 mm. C. B. length. Reconstruction of the median sagittal plane through the pharyngeal region, showing the large bursa, the notochord, and the persistent stalk of the hypophysis. The fascia pharyngobasilaris is also indicated. $\times 10$.

transection; see description of figure) in a high-power drawing, figure 7. Although the origin of the bursa pharyngea in the human embryo is undoubtedly closely bound up with notochordal contact, still the picture suggests that the development of the bursa is due to the tension exerted not merely by the notochord alone, but equally by the sheath of developing connective tissue about the notochord. This sheath of connective tissue is later incorporated with the fascia pharyngobasilaris. It is possible, however, that notochordal contact may influence the mesenchyme to condense earlier to form the sheath, since



Fig. 7 Human embryo, 46 mm. C. B. length (no. 93Hb., Cornell Collection) twin to the embryo from which figure 6 was drawn, cut in the horizontal plane. The bursa pharyngea is shown cut nearly transversely. A portion of the basilar plate is shown. To show the relation of the bursa to the surrounding connective tissue. $\times 100$.

the fibers seem to appear in the human embryo at a relatively earlier stage than in the calf and the pig.

In conclusion, it is advisable to emphasize again the mechanical nature of the pharyngeal outpocketings in the calf (when present) pig, and human embryos. These structures are merely mechanical expressions of the growth conditions of the pharyngeal region, arising in accordance with the presence of the mechanical factors determining them. The bursa pharyngea of man therefore cannot be considered a structure of fundamental significance, essential to the development of the pharyngeal tonsil,

as Schwabach maintained. If it were essential, its presence might reasonably be expected in the pharyngeal roof of all forms which possess a well-developed pharyngeal tonsil. It has been shown that the calf is a form possessing a well-defined pharyngeal tonsil, but in the calf a pharyngeal pocket corresponding to the bursa pharyngea of man does not regularly develop. There is, however, one outstanding feature common to the pharyngeal region of the calf, pig, and human embryos,



Fig. 8 Calf embryo, 190 mm. total length. Drawing of a section near the median sagittal plane, showing the location of the developing pharyngeal tonsil in its relation to the fascia pharyngobasilaris, pharyngeal musculature, and pharyngeal epithelium. Glands are drawn in only as they occur in the region of the tonsil. *T.*, pharyngeal tonsil; *L.*, larynx; *E.*, esophagus. $\times 10$.

namely, a close relationship between the fibers of the fascia pharyngobasilaris and the pharyngeal epithelium.

Figure 8 of the pharyngeal region of a 190-mm. calf embryo shows the pharyngeal tonsil developing as diffuse tonsillar tissue in the pharyngeal mucosa, over an area roughly coextensive with the terminations of the radiating fibers of the fascia pharyngobasilaris.

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Resumido por el autor, C. V. Morrill.

Reversión de la simetría e imágenes reflejadas en los monstruos de trucha, con una comparación de condiciones semejantes en los monstruos dobles humanos.

En una serie de truchas recién salidas del huevo, que contenía muchas monstruosidades dobles del tipo dicéfalo, el autor ha encontrado un cierto número en el cual las vísceras abdominales de uno de los componentes estaban invertidas simétricamente en cierto grado, produciendo de este modo una imagen comparable a la originada por reflexión en un espejo. El autor describe estas formas comparándolas con monstruos humanos dicéfalos que presentan la misma condición. También describe un nuevo caso de mónstruo humano del tipo *dicephalus tribrachius* dipus con vísceras reflejadas y dá una lista de mónstruos semejantes tomada de la literatura sobre este punto. Discute con algún detenimiento las cuestiones generales de reversión de simetría y producción de imágenes reflejadas, junto con algunas consideraciones sobre la relación de la asimetría visceral y el patrón de la segmentación.

Translation by José F. Nonidez
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SYMMETRY REVERSAL AND MIRROR IMAGING IN MONSTROUS TROUT AND A COMPARISON WITH SIMILAR CONDITIONS IN HUMAN DOUBLE MONSTERS

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EIGHT FIGURES (FOUR PLATES)

In the extensive literature on the development of monsters there are frequent references to reversed symmetry and mirror imaging of unpaired organs. This latter condition is most frequently seen in double monsters of the 'dicephalous type, one component of the monstrosity often exhibiting a partial or complete situs inversus viscerum. As far as I am aware, mirror imaging in the viscera has only been found, or at least described, in human monsters, although there is no morphological reason why monsters in the lower vertebrates should not occasionally exhibit this condition. Recently an opportunity occurred to examine the question. In a collection of newly hatched trout¹ containing many double monsters a number were found in which the abdominal viscera of one component showed reversed symmetry in some degree. The mirror imaging was practically perfect in some cases, while in others it was only slightly indicated or irregularities appeared which made the interpretation difficult. The conditions found in these fish, although not entirely novel, seem sufficiently interesting to merit a brief description. Their theoretical bearing will be considered in conjunction with similar conditions in higher forms.

¹ I am indebted to Professor Stockard for the use of this material which he has under investigation from a somewhat different standpoint.

SYMMETRY REVERSAL IN MONSTROUS FISH

The collection of monsters under consideration exhibited various degrees of doubling. The less pronounced cases showed externally a double-headed condition with the rest of the body single. At the other extreme were duplicate twins attached face to face on a single yolk. Between were the varying degrees of anterior and posterior duplicity. In the figures (pls. 1 and 2) all the specimens are viewed from the ventral or ventrolateral surface with the yolk dissected away.

The normal asymmetry of the viscera is shown in each of the twin fish of figure 6. The stomach first bends slightly to the left, then sharply to the right, passing to the pyloric end where it turns posteriorly into the intestine. The swim bladder (*S.B.*) lies dorsal to and slightly to the left of the stomach. The liver (*L.*) lies to the right and in the hollow formed by the bending of the stomach. The urinogenital system need not be considered here.

In the monsters the doubling of the viscera corresponds in degree with the external duplicity. In the specimens shown in figures 1, 2, 4, and 5 where the head and a considerable part of the trunk are double, there are two stomachs, two swim bladders, and two livers. The intestines are separate anteriorly, but unite posteriorly to form a common rectum which opens in the usual position through a single vent. Also, though not brought out in the figures, there are two hearts and two complete pairs of pectoral fins. In the specimen shown in figure 3, the duplicity does not extend as far posteriorly as in the foregoing. In this case there are two stomachs and two swim bladders, but the intestines are united immediately beyond the stomachs into a common bulbous enlargement (*C.I.*) from which a single tube leads straight backward to the vent. The liver (*L.*) is a single, irregular mass nearly twice the size of a normal liver and probably formed by the union of two separate liver buds. It lies on the right side of the monster considered as a unit. The swim bladder corresponding to one of the components (lower in the figure) passes backward on the dorsal side of the liver

and is almost concealed from view. The tip of it can be seen at *S.B.* The swim bladder of the other component is in the usual position. The specimens shown in figure 6 are twins which have been dissected away from a single yolk uniting them face to face. Each has the normal complement of organs.

It will be convenient to designate the two components of a monster as A and B. The lower component in each figure is A and the upper is B. If one imagine the figures turned so that the heads are toward the top of the page then A is on the left of the observer as he faces the ventral surface of the specimen and B is on the right. The scheme then conforms to that which Wilder ('04, '08, '16) has adopted for human monsters. If figures 1, 2, and 4 are now examined, it will be seen that in each case the asymmetry of one component is the reverse of the other, as far as the principal abdominal viscera are concerned. Component B (upper or right-hand) has the normal asymmetry—the stomach bends first to the left, the swim bladder (*S.B.*) dorsal and slightly to its left, the liver (*L.*) on the right side.² Component A (lower or left-hand), however, has reversed asymmetry—the stomach bends first to the right, the swim bladder (*S.B.*) dorsal and slightly to its right, the liver (*L.*) on the left side.

The transposition of viscera in one component is practically complete in the three specimens shown in figures 1, 2, and 4. In several other specimens (not figured) a partial reversal was indicated. Figure 3 shows a case in which the doubling involves only the head and extreme anterior end of the trunk. There are two hearts, as the figure shows, and four complete pectoral fins. In the abdominal cavity the two stomachs are crowded together and very much contorted, though both seem to bend toward the same side. There is a single large liver (*L.*), probably formed by the union of two liver buds. The swim bladder of component B (upper or left-hand) is the normal position. Its mate of the opposite side, for the most part hidden by the liver,

² The rights and lefts are used here with reference to one component, not to the entire monster.

does not exhibit the relations to be expected if mirror imaging were present, as a comparison with the position of the swim bladder in the corresponding component of figures 1 and 2 will show. It is difficult to draw any positive conclusion from this specimen, owing to the crowded condition in the abdominal cavity; but from the position of the stomachs and swim bladders, I am inclined to think that no reversed asymmetry is present in either set of organs.

It is to be noted that the monsters showing complete reversal of symmetry on one side are all in the same stage of duplicity (figs. 1, 2, and 4). No sign of reversal was found in the more complete stages of doubling. In the twins from the same egg (fig. 6) each has the same (normal) asymmetry. In the case described in the preceding paragraph, where the degree of doubling was less marked (fig. 3), all indications were contrary to the idea of symmetry reversal.

The mirror imaging described above, whether complete or partial, is not by any means the rule in these monsters. Even in the particular stage of doubling in which it occurs the majority of the specimens show the normal asymmetry in both sets of visceral organs. Figure 5 illustrates this latter condition. Here it is obvious that both stomachs bend first toward the left, forming a bay, which opens toward the right. The liver (*L.*) lies in the bay; that is, on the right side of the component to which it belongs. The swim bladders (*S.B.*) lie dorsal and slightly to the left of their respective stomachs. Summarizing the facts briefly, the abdominal viscera are mirror images of each other in some cases (figs. 1, 2, and 4) and not in others (fig. 5), though most of the specimens present the same degree of doubling. Discussion of this point may be conveniently postponed until the conditions in human monsters have been described.

Despite the very simple condition of the gastro-intestinal tract in fish, the normal asymmetry is very well marked, and any change from this strikes the eye immediately upon opening the abdominal cavity. In view of this, it is curious that neither Windle ('95) nor Gemmill ('01, '12) observed any reversal of sym-

metry in their work on monstrous fish. Gemmill especially, in his study of the anatomy of double monstrosities in trout ('01), made a careful examination of the internal organs, but apparently saw no changes in symmetry, though some of his specimens exhibited the same degree of duplicity as those described in the present paper. Possibly reversal of symmetry is rare in fish, but the total number of specimens examined is too small to form any definite conclusion on this point.

SYMMETRY REVERSAL IN HUMAN MONSTERS

It is well known that some types of human diplopagi exhibit symmetry reversal and consequent mirror imaging in the unpaired viscera. At the time the present study was begun my attention was drawn to a double-headed human monster which had been kept in a museum jar for a number of years in our laboratory. It belongs to the dicephalous variety, which is the one most likely to show mirror imaging in the viscera, judging by previous reports.³ A photograph of the monster is shown on plate 4 (fig. 8). Using Fisher's ('66) classification, it would be a *dicephalus tribrachius dipus*. The name is so descriptive that nothing more need be said of the external configuration. It is similar to the Barkow fetus, no 66 in Fisher's list, except that in the present specimen the hands of the median arm are placed palm to palm and the heads are somewhat nearer together. The sex, as in Barkow's case, is female.

The organs of the upper abdomen, with the exception of the liver, show complete mirror imaging (fig. 7). The stomachs are placed with the pyloric ends pointing toward each other. There are two spleens (*Sp.*), two gall-bladders (*G.B.*), two common bile-ducts, and two pancreases (*Pan.*), each set showing the proper relations to the corresponding stomach. (The hepatic ducts are not shown in the diagram.) The liver forms a large compound mass with many irregular lobules (not figured). The small intestines are separate to within two feet of the caecum, at which point they unite to form a common ileum. The

³ Fisher ('66); Eichwald ('70); Hirst and Piersol ('93).

large intestine, including caecum (*Cae.*) and appendix, is single. The caecum lies in the right iliac fossa, from which the colon (*A.C.*) ascends in the usual way to the liver. From here the transverse colon crosses to the hypochondrium of the opposite side, the splenic flexure lying in relation to the spleen of the B-component (right-hand, as one faces the monster). The descending colon has the usual course and relations. There is a single pair of large, lobulated kidneys, each with a normal ureter. The uterus, tubes, ovaries, bladder, and rectum are normal in size and position for a single individual.

The viscera are shown schematically in figure 7. It will be observed that the organs of the right-hand component (B) are more normal in shape and larger than those of the opposite side and that they have the normal situs. There is a disparity in size also in the thoracic organs (to be described below), again in favor of component B. The two heads, however, are practically the same size though one, again the right-hand (component B) is placed a little more in the direct line of the compound body.

The thoracic cavity contains two complete sets of organs. There are two hearts inclosed in a single pericardium. They were pressed close together, both apices directed forward and downward. In figure 7 the apices have been widely separated to show the medial surfaces of both hearts. It is obvious that there is transposition in the left-hand heart (component A). The left atrium in this case receives venous blood from the superior vena cava (*S.V.C.*) and hepatic veins (*Vv.h*) and delivers it to the left ventricle from which the pulmonary artery (*P.A.*) springs. The pulmonary veins here empty into the right atrium and the aorta (*A.*) springs from the right ventricle.⁴ The reversal of symmetry is thus complete. The right-hand heart shows the normal symmetry and need not be described. The lungs consist of two pairs corresponding to the two tracheae. Those of the right-hand, or B-component, are normal in lobu-

⁴ A detailed description of the vessels in this monster will appear in a forthcoming paper by Mr. H. B. Sutton, of our laboratory.

lation and nearly so in size, while the other pair are much reduced.⁵ There is a fair-sized thymus for each side.

The diaphragm is extremely incomplete. Both stomachs with their adnexa are herniated high into the thorax, especially in component A, the fundus of whose stomach lies almost in the root of the neck.

Summarizing the more important features of the monster: The viscera of the right-hand component (B) are the more normal in size and shape and they have the normal situs. Those of the other component (A) are, generally speaking, reduced in size or irregular in shape and display situs inversus. Externally the head and neck of component B are more nearly in line with the axis of the trunk. Obviously it is always the same component (the left-hand or A-component according to the scheme adopted in the present paper) which exhibits transposition of the viscera whether in man or in fish (compare figs. 1, 2, 4, and 7). This point will be discussed in a later part of the paper.

It has been frequently assumed, as pointed out above, that monsters of certain types, especially the dicephali and ischiopagi, may be expected to exhibit mirror imaging. The number of cases on record where actual examination disclosed this condition are however, very few,⁶ and most of them are cited by Fisher ('66) in his very comprehensive paper on diploteratology. From his list I have collected the following cases:

Dicephali

Case 50. Ritta-Christina, a dicephalus tetrabrachius dipus (female) which lived about eight months. At autopsy it was found that the pericardium was single, but enclosed two hearts, which were right and left, touching at their apices. The stomachs, spleens, and pancreases were right and left, and placed so that the pyloric ends of the stomachs faced each other, the adnexa conforming as in the specimens described above. The livers, also right and left, were fused, and there

⁵ It was impossible to determine the lobulation of this pair of lungs as they were partly destroyed when the corresponding head was removed from the body during delivery.

⁶ Bateson ('94) states that Eichwald (Pet. med. Zeitsch., 1870) found some transposition of viscera in thoracopagi, though to a varying extent. The original paper was not available to the writer.

were two gall-bladders which occupied a median position. Other details need not be given here.

Case 60. *Dicephalus tribrachius tripus* (male). This specimen had two hearts, one right and one left, in a single pericardium; two aortae, one transposed, i.e., lying on the right of the vertebral column. The liver consisted of three portions, two lateral, each of which corresponded to the right lobe of a normal liver, one of them reversed, and a median lobe corresponding to the left lobes of normal livers fused. Each lateral lobe had a bile duct, gall-bladder, common duct, and portal vein symmetrically placed. There were two stomachs with pyloric ends turned toward each other; the fundus of that belonging to 'A' was in the right hypochondrium and therefore reversed, while that of 'B' had the usual position in the left. A spleen was connected with each.

Case 71. *Dicephalus dibrachius dipus* (Gruber); sex not stated. There were two food passages; two stomachs with the fundus of each turned outward, and two intestines to within five inches of the lower end of the ileum. There was a large compound liver, two gall-bladders, and two bile-ducts; no pancreas; one spleen on left stomach; two hearts, the right small and imperfect; two sets of lungs and tracheae; urogenital organs single and normal.

Case 74. *Dicephalus dibrachius dipus* (Horner); sex, male. The thorax contained a compound heart. There were normal right and left lungs and a third compound lung due to the coalescence of adjacent lungs of different fetuses. The liver was single, but compound with increased number of lobes. The gall-bladder was double with a common duct which terminated in two orifices, one for each duodenum. There were two stomachs, one on the right, the other on the left, having their pyloric orifices pointing towards each other. The two small intestines, more or less adherent, finally blended into a single tube. The colon was single. There were two pancreases, but only one spleen, which was attached to the larger left stomach. The kidneys were a single large pair; the bladder and genitals were single.

Case 102. *Dicephalus monauchenos* (White); sex, female. There were two stomachs, the left in the usual place, the right reversed, its larger extremity towards the right. The two were united at the pylorus and opened into a common duodenum. The liver was single and very large.

One further specimen may properly be placed with the foregoing five, namely, a *dicephalus dibrachius dipus* (female) described by Fisher (case 76) *which possessed a single globular stomach with right and left fundus resulting from the fusion of two stomachs*. An oesophagus from each mouth entered the compound stomach nearly at the same point. The liver and intestines were single.

The cases cited above together with the one given in the present paper, are the only definitely described cases of mirror

imaging in the dicephali. Unfortunately, the position of the viscera is not stated in the reports of Barkow's fetus (*tribrachius dipus*) and Ruggles' fetus (*dibrachius dipus*). Both of these had two stomachs, and it seems almost certain that one was transposed. We have, then, six certain cases of transposition and one indication of this condition in the fetus having a compound stomach with right and left fundus.

Incidentally, one rather striking fact is brought out in looking over the various reports. In human monsters the amount of doubling in the viscera does not necessarily correspond with the amount of external doubling, as was the case in trout. Fisher cites one case (no. 64, from Benedina), a *dicephalus tribrachius tripus* (male) in which the gall-bladder, stomach, pancreas, spleen, and intestines were all single, although there were two hearts, two urinary bladders, and two pairs of kidneys. Compare this with the two cases of *dicephalus dibrachius dipus* (nos. 71 and 74) in which the digestive systems were double as far as the lower part of the ileum. It must be admitted that Benedina's case, if correctly reported, is very unusual.

Among other classes of diplopagi in which the two components are more widely separated, it is difficult to find definite information on the position of the viscera. Bateson ('94) quotes Eichwald (l.c.) to the effect that the thoracopagous monsters examined by him showed, in almost every case, some transposition of the viscera of one of the bodies, though to a varying extent. The pygopagous 'Carolina twins,' Millie-Christina (colored), were examined while living, and it was reported that "the apex of Christina's heart is on her left side while that of Millie is distinctly felt in the right side." Gemmill ('02) reports a case of *ischiopagus tripus* (human) in which modified transposition occurred in the liver. His figure 14 seems to indicate transposition of the thoracic viscera of one component as well, but the author does not comment on it. Windle ('94) gives a report on the 'Orissa sisters,' Radica-Doodica, who were united in the thoracic region (*xiphopagus* or *thoracopagus*). Regarding the position of the viscera, he states that authorities differ as to whether one was *situs inversus viscerum*. In the case of

the famous Siamese twins, one of them is stated to have had a partial reversal of viscera. These few reports, meager as they are, show that some trace of visceral transposition or symmetry reversal may occur in monsters other than dicephali.

In the syncephali, including Janus monsters, transposition of the viscera in one component apparently does not occur, though it seems to me in one case a slight indication was observed. Wilder ('08), in describing a case of this kind (the 'Baldwin synote') makes the following statement:— "The common oesophagus leads into a common stomach, though evidently one formed of two components, since *it presents two cardiac enlargements one on either side of the oesophagus*" (italics mine). "The outline of the stomach is thus heart-shaped, but is not quite symmetrical, since the cardiac lobe of component A is a little larger than that of Component B." With regard to the remaining organs the author states that there is no trace of 'looking-glass symmetry.' The stomach of this synote is thus similar to that of Fisher's dicephalus (case 76) mentioned above.

Among other mammals a number of syncephali have been described: kitten, McIntosh ('68); cat, Reese ('11); pig, Carey ('17), but none apparently showed any trace of mirror imaging. Kaestner ('07) has described in detail several syncephalous chick embryos, with especial reference to the heart region, but they were not far enough advanced in development to show the position of the abdominal viscera. Bishop ('08) gives an account of the heart and anterior arteries in several dicephalous reptiles, but as no pronounced asymmetry of the heart is visible in this class of vertebrates, there is little opportunity to look for mirror imaging. In cases where two hearts were present, both aortic arches developed on each side. It is unfortunate that among the large number of double monsters reported so much attention has been paid to external features and so little to the position of the abdominal viscera.

DISCUSSION

The question of symmetry reversal and mirror imaging has been discussed most recently by Wilder ('04, '16), Bateson ('16), and Newman ('16, '17). It seems to be generally agreed that transposition of the viscera does not occur in human duplicate twins. In armadillo quadruplets Newman finds, after examination of a considerable number of sets, that no symmetry reversal is present in the viscera. The same is true in the duplicate twin trout (fig. 6) described in the present paper. Some mirror imaging, however, does occur in human duplicate twins and armadillo quadruplets, but it is confined to the integumentary structures (friction-skin patterns in the former case, arrangement of the scutes and bands in the latter). The integument of young trout, unfortunately, does not present any regular pattern of asymmetry, at least none could be detected, and thus yields no information on this point. In double monsters, however, it is admitted that a certain amount of symmetry reversal in the viscera is to be expected, although it may not occur in every case. Fisher, in 1866, clearly expressed this opinion, and is quoted by Wilder ('04) to this effect. Wilder, though also quoting Bateson's ('94) opinion, in agreement with Fisher, seems unwilling to admit the importance of this phenomenon and gives little space in his earlier paper (l.c.) to its discussion. In a later paper ('16), however, he discusses a very interesting case of mirror imaging in the friction-skin patterns of a human diplopage.

Newman, ('16, '17) has given perhaps the fullest discussion of symmetry reversal, both in multiple births and in monsters. The relations of symmetry observed in armadillo quadruplets are, he considers, "the results of an intricate interplay of three grades of successively operating symmetry systems, the later tending to obliterate the effect of the earlier, but not always successfully." This conclusion is based on the nature of the polyembryonic development observed in these animals and is explained by Newman as follows: "When the primary outgrowths are formed (i.e., fission in the blastocyst stage), they are the product of the antimeric halves of the first embryo and should

therefore show mirror-image relations. But a partial physiological isolation of the two halves permits a certain reorganization, or regulation of new symmetry relations, which tends more or less completely to destroy the original symmetry, yet often leaving a trace of the latter. Similarly, when the secondary outgrowths arise between the primary ones a certain residuum of the primary symmetry may be carried over that frequently manifests itself in mirror imaging between twins derived from one-half of the original embryo. Finally, when each secondary outgrowth organizes its own bilateral symmetry, it tends to lose, partially at least, the earlier symmetry relations and to establish its own mirror imagings of right and left sides" (third grade of symmetry). It must of course be remembered that in armadillos, mirror imaging between twins is confined to integumentary structures. In the case of duplicate twins and double monsters, there would be according to Newman's conclusion, only two 'grades of symmetry systems.' Any mirror imaging present in a monster would thus be evidence of the potency of a primary symmetry which had not been overcome by the secondary symmetry acquired later by the separate components. If physiological isolation occurs in a comparatively early stage, there will be, he thinks, very little mirror imaging, as the secondary symmetry will have more time to operate. Conversely, if it appears somewhat later, there will be more mirror imaging. In consequence, double monsters probably arise somewhat later in ontogeny than duplicate twins, since the former more often show evidence of mirror imaging.

Newman's suggestion regarding primary and secondary symmetry systems is to some extent supported by the conditions found in trout monsters. However, by far the greater number of these monsters, of whatever degree of doubling, show no influence of a primary system of symmetry, that is a symmetry of the monster taken as a unit. On the contrary, each component develops its own system (secondary, according to Newman) as if it were entirely disconnected from its mate (fig. 5), and this symmetry (asymmetry), moreover, is the same as that of a normal fish. It is interesting to note that in the type of

double monster known as autosite-and-parasite, a number of which occurred in the present collection, the parasite, whenever it was of sufficient size to possess a complete set of abdominal organs, always exhibited its own (secondary) symmetry and never appeared as a mirror image of the autosite. It is only in a small proportion of the monsters that the primary symmetry of the whole is still potent, in which case mirror imaging appears in the viscera (figs. 1, 2, and 4).

Newman's further suggestion that there is a direct relation between the occurrence of mirror imaging and the period in ontogeny at which doubling takes place, does not accord with what seems to be the mode of origin of monsters in fish. In this form, the initial doubling probably always occurs at the same period of development, regardless of the degree of separation of the two components. This period corresponds with the first appearance of the embryonic anlage at the circumference of the blastoderm, as Kopsch ('99) concluded in his analysis of the causes of fish monsters.⁷ In the case of double monsters, two embryonic anlagen are formed at the same time. The degree of doubling will then depend on how near the two anlagen lie to each other. On this view, mirror imaging and the time at which doubling first appears cannot be causally related. Nor is there a very precise relation, it seems to me, between the amount of separation of the two components and the occurrence of mirror imaging. It is true that there is a stage of doubling more favorable than others for exhibiting symmetry reversal in one component, but only a small proportion of the monsters even then show any evidence of this condition (compare figs. 4 and 5). Furthermore, specimens showing less separation than in the stage just mentioned might be expected to exhibit more evidence of primary symmetry (symmetry of the monster as a whole) and therefore more mirror imaging, while in point of fact the contrary is true (p. 268).

It was pointed out (p. 271) that in both fish and human monsters it is always the same component (the left-hand or A-com-

⁷ This view apparently originated with Lereboullet. Kopsch has developed it in considerable detail in the paper referred to above.

ponent) which exhibits transposition of the viscera. In this the writer agrees with Eichwald, as quoted by Bateson ('94), except that the latter uses the term 'right twin' for what is here called left-hand or A-component. Bateson himself is in doubt on this point and quotes Küchenmeister⁸ to the effect that in xiphopagous twins it may not be possible to say which is the right and which the left. This objection, however, does not apply to dicephalous forms, whether fish or human. Here the undivided portion of the monster obviously has dorsal and ventral surfaces and these may be traced without interruption into the corresponding surfaces of the two components which usually face each other to some extent. The right-hand and left-hand components are thus easily distinguished. In cases where mirror imaging occurs, the arrangement of the two sets of organs is always the same⁹—the stomachs bend first toward the lateral borders of the monster (taken as a unit), then toward the median plane (plane of union) so that their pyloric ends face each other; the livers lie close together or are fused. It is difficult, however, to find an explanation for this fact, for even if the reverse arrangement occurred, there would still be mirror imaging—the fundus of one stomach facing that of the other, the pyloric ends pointing in opposite directions, the livers lying on the lateral borders of the monster. It has sometimes been assumed that in normal development the direction of growth taken by the liver bud determines the plan of asymmetry of the remaining viscera. In the case of monsters having either two livers or a composite liver, it might be further assumed that the two liver buds, having formed independently, were drawn together by some sort of mutual attraction. If such a movement took place, the anterior ends of the two intestines together with

⁸ Die. angeb. Verlagerung d. Eingeweide d. Menschen, Leipzig, 1883. The original was not available to the writer.

⁹ An exception to this appears to have been found in the famous Siamese twins where it was Chang, the left twin (right-hand or B-component of the present paper), in whose body there were indications of situs inversus (Küchenmeister, quoted from Bateson). This would give the converse of the usual arrangement. The writer has not had access to the original description of these interesting twins.

the pyloric ends of the stomachs would be drawn with the livers toward the plane of union. This would result in the arrangement found in practically all monsters in which mirror imaging occurs. While the above assumptions do, to some extent, account for the facts, there is some evidence to show, as will be pointed out below, that the factors controlling asymmetry are located in the primitive gut and become operative before the liver bud has developed.

It must be admitted that we are still in the dark regarding the causal factors underlying the conditions of mirror imaging found in some types of monsters. The question here arises, why, in a certain stage of doubling, should mirror imaging occasionally appear and not always? One might assume that the rate of development in one component of a monster occasionally becomes a little slower than in its mate so that it tends to fall behind and is unable to develop or express an independent system of symmetry like that of a normal embryo. In this case the lagging component might be thought of as sharing with its more vigorous mate in a single system of symmetry, that is, the symmetry of the monster taken as a unit. The result would then be a mirror-imaged condition of the viscera. A suggestion of inferiority in one component was noted in the human monster described above where the transposed set of organs were found to be slightly smaller and more irregular in shape than those of the opposite side. In the fish monsters, however, no such inequality between the two sets of organs was observed. Furthermore, the assumption that a retardation of development in one component predisposes to transposition of viscera is rendered improbable by the conditions found in monsters of the autosite-and-parasite type. Here it may be fairly assumed that the parasite tends to be weaker than the autosite, and in fact is often defective; still whatever asymmetry exists in the parasite is that of a normal fish and never reversed. In other words, the parasite, even in its failing struggle for existence, retains the power to develop its plan of asymmetry as a separate individual.

It is extremely difficult to formulate a theory which will satisfactorily account for a condition so casual in its appear-

ance as mirror imaging in the viscera of monsters, and further work on the early developmental stages of these forms is necessary before any definite conclusions can be drawn. The solution will, of course, involve the more fundamental problem of what determines the normal asymmetry of unpaired organs and why single individuals occasionally appear with transposed organs.¹⁰ Very little progress has been made in this direction. The most suggestive observations in the field are those of Pressler ('11), on experimentally produced situs inversus in *Bombinator*. The material was obtained from Spemann who performed the following experiment: In the neurula stage, a four-sided piece of the medullary plate together with a portion of the roof of the primitive gut lying under it was cut out and replaced in reversed position, so that the anterior extremity of the piece was directed posteriorly, the posterior extremity, anteriorly. From these experimental embryos, tadpoles were reared which showed in many cases a complete situs inversus viscerum. It has sometimes been assumed, as stated above, that the asymmetrical growth of the liver bud normally toward the right influences the position of the remaining organs. The question then arises, what determines the direction of growth of the liver bud? Spemann and Pressler's work seems to indicate that the factors controlling asymmetry are located in the primitive gut and probably arranged in such a fashion as to cause the gut in normal development to bend first toward the left, thus forcing the liver bud to grow toward the right. We may suppose that when the arrangement of these factors is reversed, as in the experiment,

¹⁰ Bateson ('94, p. 560) points out that cases of this kind cannot be explained on the ground that one member of duplicate twins has died or failed to develop, since it has been shown that in duplicate twins neither member has transposed viscera. Conversely, Küchenmeister (l.c.) collected 152 cases of transposition, of which only one could be shown to have been a twin.

A somewhat similar suggestion has been made to account for cases of situs inversus in single individuals, namely, that this condition results from complete reduction of one component of a monster (autosite- and-parasite) in which mirror imaging occurred. The autosite in this instance must necessarily present the reversed asymmetry. In some cases it is thought that the parasite is taken into the body of the autosite during development, and gives rise to certain kinds of tumors. As far as I am aware, there is no evidence recorded that individuals with complete situs inversus have possessed tumors of this sort.

transposition is produced. Pressler's observations are, it seems to me, very important and indicate the direction along which further experiments should be made to determine the cause of asymmetry. They do not, however, throw any light on the cause of transposition in integumentary structures as found by Newman and Wilder.

A very interesting suggestion as to the cause of asymmetry in the viscera is based upon the fact, first pointed out by Crampton ('94), that in certain gasteropods the position assumed by the adult organs is correlated with the early segmentation pattern. In these snails the more usual type of asymmetry with dextral shell is associated with a right-handed spiral cleavage. Some forms, however, such as *Physa* (Crampton) and *Ancylus rivularius* (Holmes), have normally sinistral shells and reversed asymmetry in the viscera; this condition was found to be associated with a reversal of cleavage. These observations very naturally led to the view that in gasteropods there is a causal relation between cleavage pattern and the type of asymmetry found in the adult.¹¹ It is very questionable, I think, whether this conception of the primary cause of asymmetry can be applied to vertebrates. For in monsters, as has been shown, two sets of organs may develop as mirror images of each other, one with normal, the other with reversed asymmetry, though obviously both have arisen at the same period of development from a single blastoderm. It is difficult to imagine how changes in early cleavage pattern, if such occur in higher forms, could bring about the development of two types of asymmetry in the same embryo, as in the case just cited. From the evidence at hand, it seems probable that the primary cause of visceral asymmetry in vertebrates is to be sought for at the completion of cleavage rather than in the period of cleavage itself.

¹¹ This view, first expressed tentatively by Crampton ('94), was later more fully developed by Conklin ('97, *Jour. Morph.*, vol. 13) and by Holmes ('99, *Amer. Nat.*; '00, *Jour. Morph.*, vol. 16) on the basis of additional evidence. Conklin more recently (*Heredity and Environment*, 2nd ed., 1917, p. 177) has expressed the opinion that the correlation between inversion of cleavage and inversion of symmetry observed in certain snails, will be found "*probably in all animals showing inverse symmetry*" (italics mine). I do not believe this latter generalization is warranted for the reasons given in the discussion (see beyond).

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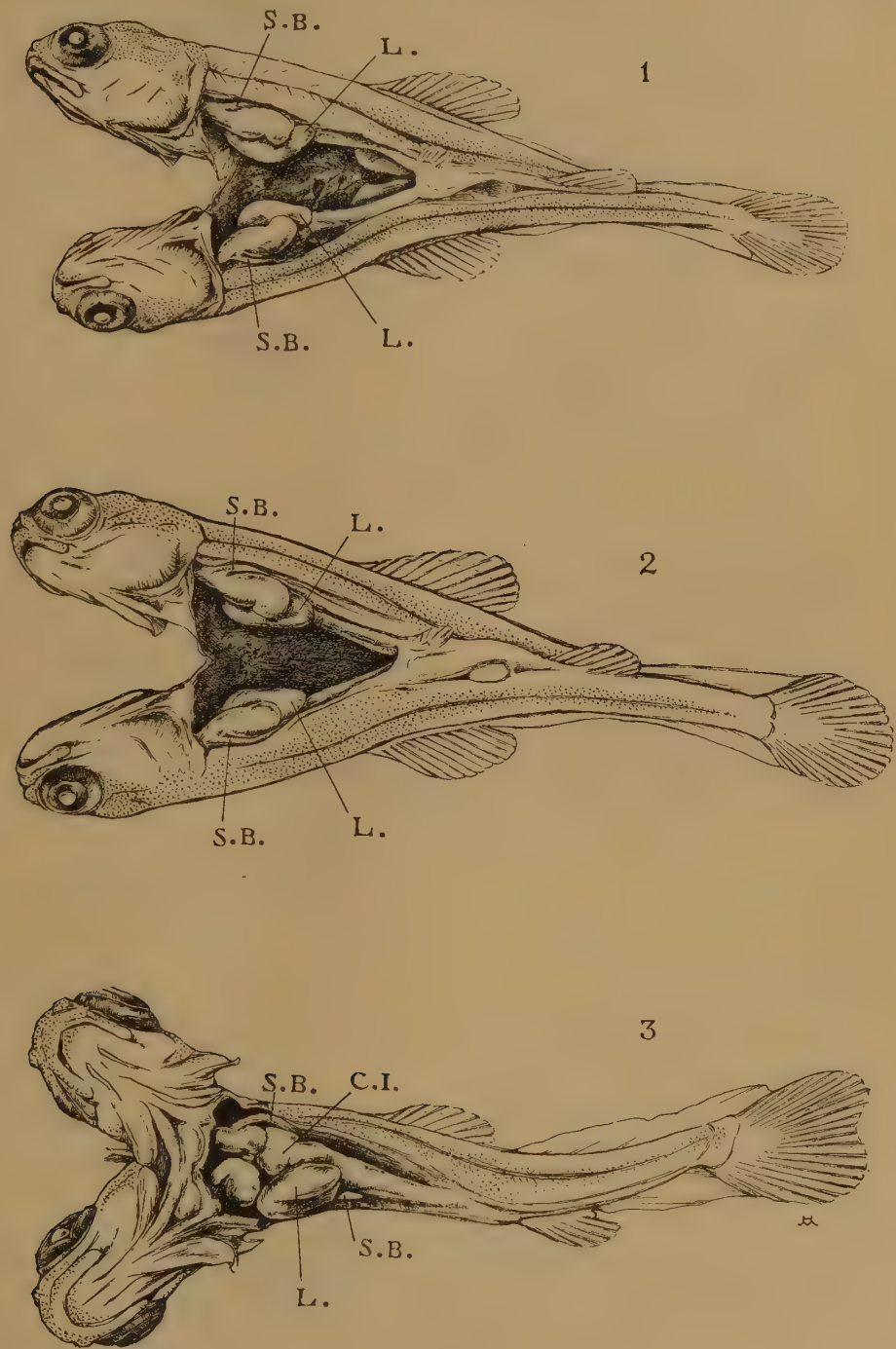
PLATES

PLATE 1

EXPLANATION OF FIGURES

1 and 2 Specimens of monstrous trout showing complete mirror imaging in the abdominal viscera, ventrolateral view. *S.B.*, swim bladder; *L.*, liver; the stomach and intestine are not labeled. The position of the viscera in one component is the reverse of that in the other.

3 Specimen in which doubling is less extensive than in the foregoing (1 and 2). There are two stomachs and two swim bladders (*S.B.*) one of which is almost concealed by the compound liver (*L.*) The intestines unite immediately beyond the stomachs into a common enlargement (*C.I.*). Apparently no mirror imaging is present in this case. The two pear-shaped bodies anterior to the abdominal cavity are the hearts.



H. Murayama, del

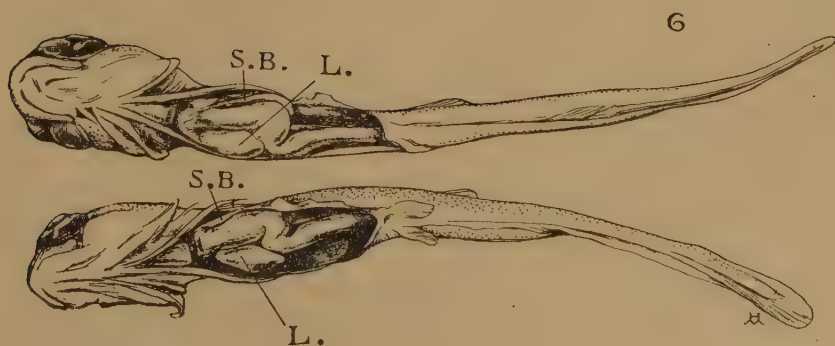
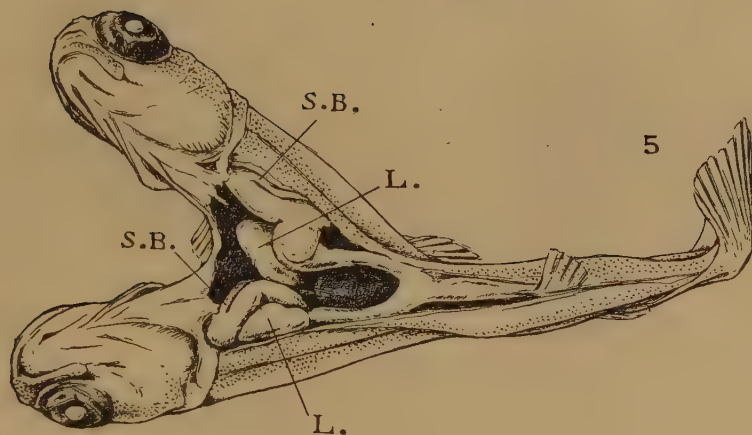
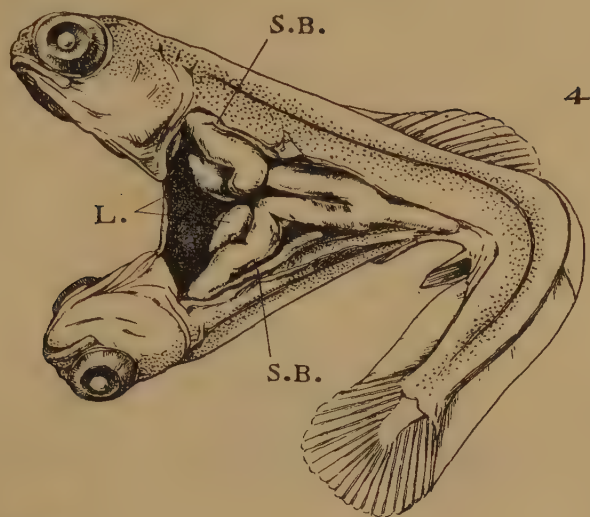
PLATE 2

EXPLANATION OF FIGURES

4 Specimen showing complete mirror imaging similar to figures 1 and 2 except that the two sets of organs are closer together, the livers (*L.*) almost in contact; ventrolateral view.

5 Specimen showing the position of the viscera in the majority of monsters in this stage of doubling. The normal situs is present in both components; ventrolateral view. Compare with figure 4.

6 Twins from the same egg. These two specimens lay on opposite sides of a single yolk mass. The position of the viscera is the same in both, the liver on the right, the stomach bulging toward the left as in normal fish (i.e., normal situs in both).



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PLATE 3

EXPLANATION OF FIGURES

7 Diagram of the viscera in the human monster shown in figure 8. The compound liver is omitted. The apices of the two hearts are widely separated to show the medial surfaces which were in close contact. *A.*, aorta; *P.A.*, pulmonary artery (origin); *S.V.C.*, superior vena cava; *Vv.h.*, hepatic veins; *Sp.* spleen; *Pan.*, pancreas; *G.B.*, gall-bladder; *A.C.*, ascending colon; *Cæ.*, caecum

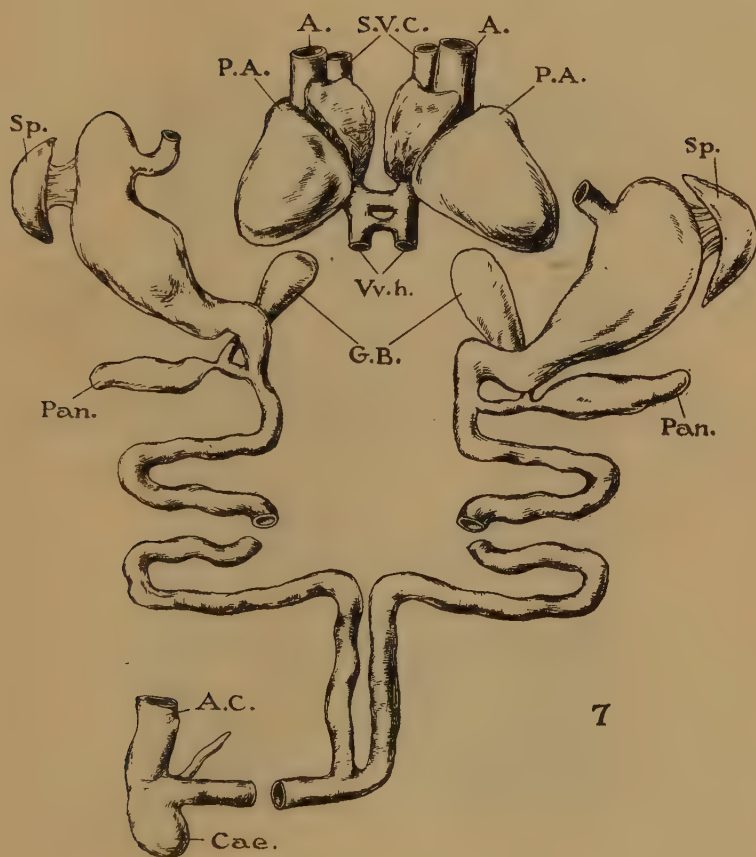


PLATE 4

EXPLANATION OF FIGURE

8 Photograph of the human dicephalus tribrachius dipus described in the present paper.



Resumen por el autor, Edward Phelps Allis, Jr.
Mentone, Francia.

La innervación de los músculos intermandibulares y geniohioideos
de los peces óseos.

El músculo geniohioideo, llamado así con propiedad, está innervado exclusivamente por el trigémino en los diversos peces óseos examinados. En ciertos teleósteos una parte del llamado geniohioideo en las descripciones corrientes, está innervada por el nervio facial y de aquí ha nacido la suposición de que este músculo tiene una doble innervación, representando un músculo, derivado de un segmento del cuerpo, que está adquiriendo innervación por el nervio de otro segmento. Esta suposición es, sin embargo, errónea, porque la parte del geniohioideo innervada por el facial se ha diferenciado del músculo hiohioideo y en los adultos de *Esox*, *Scomber*, *Gadus* y *Silurus* se encuentran estructuras que representan estados sucesivos de su desarrollo. El llamado geniohioideo en estos peces se forma por consiguiente a expensas de dos músculos claramente diferentes, uno derivado del miotomo mandibular y el otro del miotomo hial y las innervaciones presentes actualmente son primarias y no secundarias. La parte derivada del miotomo hial está colocada exteriormente a los radios branquióstegos y, a causa de esta posición y su derivación del hiohioideo, puede llamarse hiohioideo superficial. A veces forma un músculo continuo con el geniohioideo superior pero en todo caso, en los ejemplares examinados, está separado de este por una fascia membranosa o una aponeurosis transversa.

Translation by José F. Nonidez
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THE INNERVATION OF THE INTERMANDIBULARIS AND GENIOHYOIDEUS MUSCLES OF THE BONY FISHES

EDWARD PHELPS ALLIS, JR.

Menton, France

ONE FIGURE

The so-called musculi intermandibularis and geniohyoideus of Vetter's ('78) descriptions of the bony fishes are among those muscles of vertebrates that are frequently said to vary greatly in the manner of their innervation, Holmqvist even saying ('11, p. 68), in a work relating especially to them, that their innervation is so variable that it has no morphological significance whatever. Work that I have under way on the cranial anatomy of *Polypterus* having led me to doubt this statement, I have had the innervation of these muscles carefully traced not only in this fish, but also in several of the Teleostei. The work on the adult *Polypterus* was done by my assistant Mr. Jujiro Nomura, the work on the other fishes by Mr. John Henry.

In *Polypterus* there is no muscle that corresponds, topographically, to the intermandibularis of Vetter's descriptions of other fishes, but there are two muscles that correspond to the superior and inferior divisions of the geniohyoideus of that author's descriptions of those fishes, and that strikingly resemble, in their topographical relations, the two so-named muscles of my descriptions of *Amia* (Allis, '97). These two muscles of *Polypterus* were first described by Pollard ('92), and were called by him the intermaxillaris anterior and intermaxillaris posterior, some fibers of the latter muscle being said by him to be continued onward, as intrinsic muscles, into the mantle flap. Another and separate muscle, which corresponds to the hyohyoideus inferior of Vetter's descriptions of other fishes, is called by Pollard both the mantle

muscle and the muscle of the jugular plate, and it also is said to send some fibers into the mantle. The intermaxillaris anterior (geniohyoideus inferior) is said by Pollard to be innervated by a branch of the ramus mandibularis trigemini, the intermaxillaris posterior (geniohyoideus superior) and the mantle muscle (hyohyoideus inferior) both being innervated by branches of the ramus hyoideus facialis.

Holmqvist, in 1910, briefly describes these muscles of *Polyp-terus*, but he calls the intermaxillaris anterior of Pollard's descriptions the intermandibularis, and the intermaxillaris posterior the protractor hyoidei. The fibers of this latter muscle are said to run insensibly into those of the hyohyoideus, without apparent limiting boundary, this latter muscle being the mantle muscle of Pollard's descriptions. The innervation of these muscles of *Polypterus*, individually, is not given by Holmqvist, but it is said that, in the bony fishes in general, the intermandibularis and the anterior portion of the protractor hyoidei are innervated by the trigeminus, and the posterior portion of the latter muscle by branches of the nervus facialis, and it is evident that this is intended to apply to *Polypterus* as well as to the *Holostei* and *Teleostei*. That part of the protractor hyoidei of all these fishes that is innervated by the facialis is said to be its most primitive (älteste) portion, the part innervated by the trigeminus being a later acquisition (ein späterer Erwerb).

In a later work, Holmqvist ('11) says that the intermandibularis and protractor hyoidei of all of the bony fishes are derived, respectively, from the mandibular and hyal portions of the primitive musculus constrictor ventralis. The primitive condition of the intermandibularis is said to have been that of a muscle extending transversely from one ramus of the mandible to the other, and this condition is said to be actually found in the *Selachii*, in *Lepidosteus*, and in certain of the more primitive *Teleostei*. In the remainder of the *Teleostei*, and in *Amia*, the muscle is said to have undergone a vertical cleavage into two parts, one of which is called the intermandibularis I and the other the intermandibularis II, these two muscles of *Amia* being the intermandibularis and geniohyoideus inferior of my descriptions of that fish.

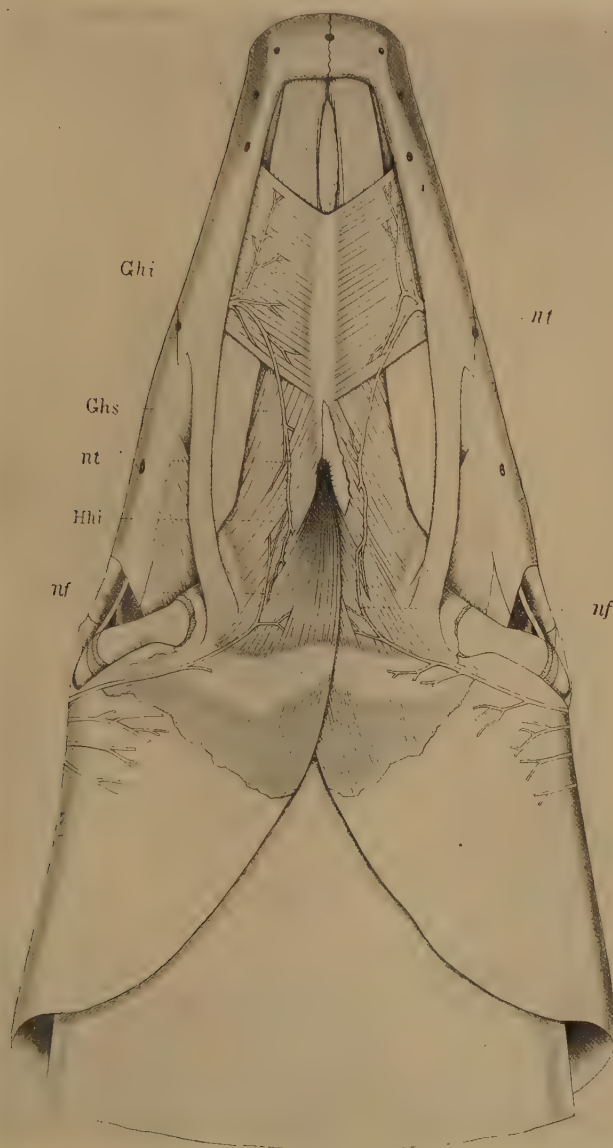


Fig. 1 Ventral view of the head of *Polypterus*, the skin removed so as to show the geniohyoideus and hyohyoideus muscles. *Ghi*, musculus geniohyoideus inferior; *Ghs*, musculus geniohyoideus superior; *Hhi*, musculus hyohyoideus inferior; *nt*, branch of nervus trigeminus; *nf*, branch of nervus facialis.

These two so-called intermandibularis muscles are said by Holmqvist to both be innervated, either wholly or in part, by a branch of the trigeminus which he calls the ramus mylohyoideus. The protractor hyoidei is said to be innervated by the ramus hyoideus facialis, and to be derived from that deeper layer of the constrictor ventralis of the Selachii that has its insertion on the ceratohyal. Holmqvist then says (l.c., p. 70) that these muscles of the Ganoidei and Teleostei have, like the corresponding muscles of the Selachii, a double innervation, by the nervi trigeminus and facialis, and that the extent to which they are innervated by one or the other of these two nerves varies greatly, and he attributes this variation, not to the abortion of any portion of the muscle fibers concerned, but either to the abortion of certain fibers of one of the two nerves, and the secondary innervation of the muscle fibers that they are primarily innervated by fibers of the other nerve, or to a change of course of certain motor fibers of the facialis, those fibers abandoning their normal course to follow the path of the trigeminus, and then gradually forcing the fibers of the latter nerve away from the muscle fibers that they primarily innervated, and there supplanting them.

Edgeworth ('11) also discusses these muscles of *Polypterus*, and he introduces still another name for one of them, calling the intermaxillaris anterior of Pollard's descriptions the intermandibularis, and the intermaxillaris posterior the hyomaxillaris. Like Holmqvist, whose works he evidently had not seen, he says that the intermandibularis (geniohyoideus inferior) is derived from the mandibular myotome, and although its innervation in *Polypterus* is not particularly given, he doubtless considered it to be by the nervus trigeminus, for he says that this muscle is usually, in the numerous vertebrates considered by him, innervated by that nerve. The hyomaxillaris is said to be the homologue of the geniohyoideus superior of my descriptions of *Amia*, to have been developed from the hyal myotome, and to have been primarily innervated by the nervus facialis; and, in a footnote (l.c., p. 210), it is said that the term geniohyoideus is avoided because it "is generally used to denote the anterior element of the hypobranchial spinal muscles." It is said that, in the adults

of the many vertebrates considered by him, the innervation of the intermandibularis and the hyomaxillaris varies considerably, the intermandibularis sometimes being wholly or in part innervated by the facialis, and the hyomaxillaris sometimes in part by the trigeminus.

Luther ('13) also refers to these muscles of *Polypterus*, and he adopts the term intermandibularis for the intermaxillaris anterior of Pollard's descriptions, but calls the intermaxillaris posterior the musculus C_{2vh} . In a figure of the adult *Calamoichthys* he shows the former muscle innervated by a branch of the trigeminus and the latter muscle by a branch of the facialis, this thus doubtless being the innervation that he ascribes to the muscles of *Polypterus*.

In an adult specimen of *Polypterus bichir* I find these muscles as shown in the accompanying figure, and it seems to me best, for the present, to still retain for them the time-honored names given by Vetter to the corresponding muscles in other fishes, notwithstanding that the term geniohyoideus is evidently inappropriate. The geniohyoideus inferior (intermaxillaris anterior, intermandibularis) is as described by Pollard and Holmqvist, but it cannot be said to be greatly reduced (*sehr reducirt*, Holmqvist). The geniohyoideus superior (intermaxillaris posterior, protractor hyoidei) is not continuous, posteriorly, with the hyohyoideus inferior, as both Pollard and Holmqvist say that it is, but the mesial edge of the posterior portion of the geniohyoideus is contiguous with the lateral edge of the hyohyoideus, the two muscles there forming a continuous sheet, so that it is difficult to tell exactly where one ends and the other begins. Anteriorly the two muscles are wholly distinct, the geniohyoideus superior lying superficial (ventral) to the hyohyoideus inferior and separated from it by a fold of the dermal tissues. The geniohyoideus superior arises from the proximal (posterior) end of the ceratohyal, and its fibers run anteromesially and diverge somewhat. The anterolateral fibers pass internal (dorsal) to the geniohyoideus inferior and are inserted on a dorsal extension of the median raphe of the latter muscle, the posteriomesial fibers being inserted on a posterior continuation of the same raphe. The anterior

portion of the mantle muscle of Pollard's descriptions is the hyohyoideus inferior.

The so-called ramus mylohyoideus trigemini of Holmqvist's descriptions issues from the ramus of the mandible onto the ventral surface of the geniohyoideus inferior, and there separates into anterior and posterior portions, both of which send branches into the muscle to innervate it. The posterior branch then continues onward beyond the hind edge of the geniohyoideus inferior, onto the ventral surface of the geniohyoideus superior, and sends branches into the latter muscle, the terminal branch of the nerve either lying close to, and parallel to, a branch of the ramus hyoideus facialis, or anastomosing completely with that branch so that it appears to run directly into it; these two conditions being found, one on either side of the head, in the single specimen used for the accompanying figure.

The dissections of this adult specimen thus show that the greater part, at least, of the geniohyoideus superior must be innervated by the nervus trigeminus, and that if any part of it is innervated by the nervus facialis it is only a few posteromesial fibers.

In a 75-mm. specimen of *Polypterus senegalus*, examined in serial transverse sections, the two branches here under consideration of the nervi trigeminus and facialis do not anastomose with each other on either side of the head, and the branch of the trigeminus sends branches into both divisions of the geniohyoideus, unquestionably innervating them. The main branch of the hyoideus facialis runs anteriorly between the geniohyoideus superior and the hyohyoideus inferior, and goes wholly to the latter muscle and to tissues of the region. A small branch perforates the posteromesial edge of the geniohyoideus superior, and, passing through it, without sending any perceptible branches to it, goes to tissues that lie internal to it, this branch evidently being that branch of the hyoideus facialis of the adult that runs anteriorly onto the ventral surface of the geniohyoideus superior and there, on one side of the head of the adult specimen above described, anastomoses with the terminal branch of the nervus trigeminus.

The conditions in this embryo thus show, even more positively than those in the adult, that the two divisions of the geniohyoideus are both innervated by the nervus trigeminus, and by that nerve alone. The posterior division of the muscle could not then have been derived from the hyal myotome, as Holmqvist and Edgeworth both maintain, unless it had, in stages younger than that represented in my 75-mm. specimen, lost its primitive innervation by the nervus facialis and secondarily acquired innervation by the trigeminus. This I greatly doubted, but it evidently would be confirmed if, as is frequently stated, these muscles are, in certain of the Teleostei, in part innervated by the nervus facialis. I accordingly had certain of these latter fishes examined, and was surprised to find that in certain of them the posterior portion of the so-called geniohyoideus superior is, in fact, innervated by the facialis. This definitely established, the muscles themselves were carefully examined and compared, and it was found that, in every case of such innervation, the fibers so innervated belonged to the hyohyoideus and not to the geniohyoideus. This was first recognized when considering the muscles in the Siluridae, and it will be best to first consider the conditions in those fishes.

In both *Ameiurus* and *Silurus* there are two muscles which McMurrich ('84), Juge ('99), Herrick ('01), and Jacquet ('01) all describe or refer to as the intermandibularis and geniohyoideus. Holmqvist ('11) calls these two muscles the intermandibulares I and II, and hence considers them to be the homologues of the muscles similarly designated by him in *Amia*, which are the muscles called by me (Allis, '97) the intermandibularis and geniohyoideus inferior. Both of these muscles are said by all these authors to be innervated by the nervus trigeminus.

The musculus hyohyoideus of these fishes is said by both McMurrich and Juge to have two distinctly different portions, and McMurrich calls them its anterior and posterior portions, and Juge its inferior and superior portions. Holmqvist calls the anterior one of these two portions of the muscle the protractor hyoidei, says that it contains no part of the hyohyoideus of other

fishes, and homologizes it definitely with the geniohyoideus superior of current descriptions of those other fishes. The so-called posterior or superior, portion of this muscle lies internal to the branchiostegal rays, but is continued forward beyond the rays, the muscle thus corresponding topographically to both the superior and inferior divisions of the hyohyoideus of *Amia*. The so-called anterior, or inferior, portion of the muscle is a thick fleshy muscle which, as shown in the figures given by authors, lies external to, and directly upon, the basal portions of the six or seven anterior (ventral) branchiostegal rays, and external, also, to that part of the hyohyoideus that corresponds topographically to the hyohyoideus inferior of other fishes. Both portions of the muscle are said by all these authors to be innervated by the nervus facialis.

In a 30-mm. specimen of *Ameiurus nebulosus*, examined in serial sections, the branches of the trigeminus and facialis here under consideration anastomose with each other as they do on one side of the head of my adult specimen of *Polypterus*. In six other specimens, varying in length from 11 mm. to 55 mm., there was no anastomosis of these nerves, and all branches of the trigeminus and facialis nerves that penetrated the muscles were distributed, respectively, to the geniohyoideus and hyohyoideus, and to those muscles only. There is thus here no possible question of a double innervation of either of these muscles, this confirming the statements made by earlier authors. That portion of the hyohyoideus that lies external to the branchiostegal rays is therefore a hyal muscle, and, because of its relations to those rays, it will hereafter be referred to as the hyohyoideus superficialis. The two other portions of the muscle will be called, as they are in other fishes, the hyohyoideus inferior and hyohyoideus superior. In the one adult specimen of *Silurus* that was examined, the anterior (ventral) branchiostegal ray on one side of the head was bent outward at its base and there perforated the hyohyoideus superficialis close to its external surface, thus practically lying external to that muscle and hence not coming into any relations whatever with the ceratohyal.

The innervation of these muscles of *Ameiurus* and *Silurus* thus showing that the *hyohyoideus superficialis* must be a muscle derived from the hyal myotome, there is evidently question as to how it could have acquired a position external to the branchiostegal rays, for those rays, being dermal structures similar in origin and character to the opercular bones, must have lain, primarily, external (morphologically anterior) to all muscles derived from the hyal myotome, and external also to all motor fibers of the *nervus facialis*. Those sensory branches of the latter nerve that were distributed to the dermal tissues on the anterior surface of the hyal arch would naturally, when the branchiostegal rays developed, have been left running outward between adjacent rays, but no motor fibers could have acquired such a course unless they had been dragged outward, out of their normal course, by migrating muscle fibers. The *hyohyoideus superficialis* of these fishes must then have acquired its actual position, external to the branchiostegal rays, either by passing outward between certain of those rays or by growing upward, external to the rays, from that part of the primitive muscle that lay anterior to the anterior ray. That the primitive *hyohyoideus* had separated into superficial and deeper portions before the branchiostegal rays were developed, and that the rays had then grown inward between those two portions and so acquired insertion on the ceratohyal, internal to the superficial portion of the muscle, seems in itself, a most improbable assumption, and, furthermore, it is not in accord with the fact above referred to that the anterior ray of my specimen of *Silurus* lies practically superficial to the superficial muscle.

If, then, the facile assumption of a secondary innervation of the *hyohyoideus superficialis* be excluded from consideration, the relations to the branchiostegal rays of the nerve or nerves that innervate this muscle of the adult should definitely show from what part of the primitive constrictor of the hyal arch it has been developed. In all my specimens of *Ameiurus* the *superficialis* muscle is innervated by a single branch of the *ramus hyoideus facialis*, and in the one adult specimen of *Silurus* this branch ran outward between the seventh and eighth branchiostegal rays,

counting upward from the ventral end of the arch. Certain fibers of the primitive constrictor must then have passed outward between these two rays, pulling with them, the nerve fibers that innervated them, and then have there developed into the large muscle actually found. If several sections of the constrictor had thus passed outward between several pairs of adjacent rays, there would have been a corresponding number of nerves innervating the superficialis muscle, and no such nerves are found. And if the superficialis muscle had grown upward from that part of the entire constrictor that lay anterior (ventral) to the most anterior (ventral) branchiostegal ray, the nerve that innervates the superficialis muscle would have first run forward (ventrally) internal to all the branchiostegal rays and then upward external to the ventral ray, and no such nerve is found.

It is thus quite certain that but a single section of the primitive constrictor of the hyal arch passed outward between the branchiostegal rays to form the *hyohyoideus superficialis*, and the point where it passed outward lies approximately between certain dorsal ones of the branchiostegal rays that have their attachments to the external (anterior) surface of the ceratohyal, and ventral ones that have their attachments to its internal (posterior) surface. This, then, at once suggested that whatever it may have been that gave rise to this arrangement of the rays had permitted, or perhaps induced, the differentiation of the *hyohyoideus superficialis*, and as this arrangement of the rays is not peculiar to the *Siluridae*, I at once reexamined my material to see if there were in any of the several specimens of the *Holostei* and *Teleostei* any indications of the differentiation of this muscle, and hence an explanation of the fact, already established, that the so-called *geniohyoideus superior* is in certain of these fishes in part innervated by the *nervus trigeminus* and in part by the *nervus facialis*.

In *Amia* the branchiostegal rays are all attached to the external (anterior) surface of the ceratohyal (Allis, '97), and there is no *musculus hyohyoideus superficialis*. In *Lepidosteus* there is also no *hyohyoideus superficialis*, but the conditions in this fish differ from those in *Amia* in that, in the single specimen examined, there were but three branchiostegal rays, and they apparently corresponded to the dorsal ones found in *Amia* and the *Telostei*.

In 37-mm. specimens of *Cottus aspera* and *Clinocottus analis* the branchiostegal rays are in part attached to the external surface of the ceratohyal and in part to the internal surface of that element, as they are in the Siluridae, but there is no *musculus hyohyoideus superficialis*. A branch of the *ramus hyoideus facialis* runs outward approximately between the rays that are attached to the external and internal surfaces of the ceratohyal, but it is not connected with the *nervus trigeminus* by anastomosis. The *musculi intermandibularis* and *geniohyoideus* are both innervated by the *nervus trigeminus*, and by that nerve alone. An artery, which arises from the so-called mandibular artery in the *ramus* of the mandible, accompanies this branch of the *trigeminus* and then the related branch of the *ramus hyoideus facialis*, and falls into an artery that runs upward in the hyal arch with the *ramus hyoideus facialis*, this artery being found in all of the Teleostei that were examined in serial sections. What the significance of this artery may be could not be determined, but it suggests the commissural vessels that, in the Selachii, connect the anterior and posterior efferent arteries of the branchial arches.

In *Porichthys notatus* there is no *musculus hyohyoideus superficialis*. In an 18-mm. specimen of this fish the two branches of the *trigeminus* and *facialis* here under consideration are not connected by anastomosis. In a 25-mm. specimen they are connected by a delicate anastomosing branch, but this branch certainly contains no motor fibers.

In an adult specimen of *Exos lucius* there is a small *hyohyoideus superficialis*, but its innervation could not be determined in the dissections. The branches of the *trigeminus* and *facialis* here under consideration anastomose with each other, as they do in *Amia* and on one side of the head of my adult specimen of *Polyp-terus*; but when the so-formed continuous nerve was treated with weak nitric acid and examined under the microscope, it was seen that there was no interchange of fibers between its two components and that it was the *trigeminus* nerve, alone, that sent branches into the *geniohyoideus*.

In *Scomber scomber* there is a fairly large *hyohyoideus superficialis*. In an earlier work (Allis, '03) I said that the *geniohyoi-*

deus superior of this fish arises by two distinctly different heads, one of which is entirely muscular and the other entirely tendinous. The muscular head was said to become tendinous slightly anterior to its surface of origin on the ceratohyal, and to there form a broad flat tendon from which the anterior and wholly muscular part of the muscle arose. I now find that this so-called tendinous part of this head of the muscle is a membranous fascia which lies external to the posterior (dorsal) portion of the muscle and internal to the anterior (ventral) portion, thus completely separating these two so-called portions of the muscle, the one from the other. In a 65-mm. specimen I find these two portions of the muscle innervated on one side of the head by anastomosing branches of the trigeminus and facialis that are strictly similar to those described by me in the adult. On the other side of the head there is no anastomosis between these two nerves, the branch of the facialis ending in the posterior portion of the muscle and the branch of the trigeminus ending in the fascia that separates that portion of the muscle from the anterior portion. The posterior portion of the muscle is thus a hyohyoideus superficialis, innervated wholly by the facialis, and the anterior portion a geniohyoideus, innervated wholly by the trigeminus.

In a 76-mm. specimen of *Caranx caranx* the conditions are similar to those in *Scomber*, but the hyohyoideus superficialis is but slightly developed.

In an adult specimen of *Pelamys* (*Scomber*) *sarda* the conditions are as in *Scomber scomber*, but the hyohyoideus superficialis has here pushed forward until it nearly reaches the level of the hind end of the geniohyoideus superior, but is there separated from that muscle by the covering fascia.

In an adult specimen of *Gadus morrhua*, I find the protractor hyoidei of Holmqvist's ('11) descriptions crossed by two aponeuroses, one lying not far from the surface of origin of the muscle on the ceratohyal and the other slightly anterior to the point where the muscles on opposite sides meet in the median line. The anterior aponeurosis is described by Holmqvist in *Gadus callarius*, but the posterior one is not mentioned by him. The anterior aponeurosis apparently separates the inferior and superior divi-

sions of the geniohyoideus, and these two parts of the muscle are both innervated entirely by the trigeminus. The posterior aponeurosis quite unquestionably corresponds to the membranous fascia above described in Scomber, and it separates the geniohyoideus superior from a hyoideus superficialis. The trigeminus and facialis nerves break up into small branches as they approach this aponeurosis, and the branches perforate the aponeurosis and anastomose with each other, but the size of the fibers, the general conditions, and, more particularly, comparison with the fishes above described, all indicate that the anastomosing branches are not motor ones. This is thus in accord with Holmqvist's statement that the posterior portion of his protractor hyoidei, which is my hyohyoideus superficialis, is innervated by the facialis and that the anterior portion of that muscle, my geniohyoideus superior, is innervated by the trigeminus. Herrick ('00) says that the intermandibularis and geniohyoideus of his fish are both innervated by the trigeminus and the hyohyoideus by the facialis; and the hyohyoideus as referred to by him certainly does not include my hyohyoideus superficialis.

The conditions in these several fishes thus show, in my opinion conclusively, that there has been no change whatever in the innervation of any of these muscles, the muscles derived from the mandibular myotome all being innervated by the trigeminus, and those derived from the hyal myotome all innervated by the facialis. The disposition of the muscles innervated by the trigeminus then shows that the ventral portion of the primitive constrictor of the mandibular arch underwent, in all these fishes, a more or less complete longitudinal cleavage, from its dorsal end downward, the dorsal end of one of these two parts acquiring insertion on the mandible and the dorsal end of the other part insertion on the ceratohyal. That part of the muscle that acquired insertion on the ceratohyal would then naturally there lie external (morphologically anterior) to the branchiostegal rays of the hyal arch, and that is, as is well known, its actual relation to those rays. The ventral end of this entire constrictor muscle must have been primarily attached either to the ventral end of the branchial bar of the mandibular arch or, and more probably,

to its fellow of the opposite side, in the median line, the fibers of the two muscles there either interdigitating with each other or being inserted in a common median aponeurosis. That part of the muscle that was inserted at its dorsal end on the mandible then frequently separated into two parts, the intermandibularis and geniohyoideus inferior, and the insertions on the mandible of both of these parts tended to shift forward (morphologically ventrally) toward the symphysis. The geniohyoideus inferior thus apparently acquired, in certain fishes, an insertion so close to the symphysis that its course was actually reversed, and it then ran from the ventral end of the arch toward its dorsal end, or, as actually found, from in front directly posteriorly. This muscle then formed with the geniohyoideus superior a single continuous muscle, the median portion of which was the primarily ventral end of the entire muscle. Frequently, however, certain fibers of the geniohyoideus superior did not have this insertion with the fibers of the geniohyoideus inferior, but passed onward internal to the latter muscle and acquired independent insertion on the mandible. Reductions or abortions of one or the other of these three muscles, or of certain parts of them, and a slight shifting of origins or insertions then gave rise to all the various arrangements actually found.

The intermandibularis and geniohyoideus of these fishes would seem to correspond, respectively, to the mylohyoideus and the anterior digastricus of mammals, and some part of the hyohyoideus to the posterior digastricus; and this, if correct, should be taken into account in any change of nomenclature that may be proposed.

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Resumen por el autor, Francis Marsh Baldwin.
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Variaciones de las arterias carótidas del conejo.

El presente estudio fué emprendido para averiguar la presencia y extensión de la variación de las arterias carótidas del conejo, habiéndose empleado 114 ejemplares, veinte y tres de los cuales (cerca del 20 por ciento) difieren de las condiciones normales descritas en los libros de texto. El autor discute las principales variaciones observadas bajo los siguientes epígrafes: Diferencias encontradas en las arterias carótido-occipitales internas, en las arterias maxilo-linguales externas, maxilo-superficiales temporales internas, temporales-occipitales superficiales y otras diferencias generales. Las relaciones de tamaño, orden de secuencia y posiciones comparadas de las diversas ramas se anotan también y discuten. En varios casos se forman interesantes arterias in-nominadas, mientras que en otros las ramas terminales forman a modo de tenedores con tres, cuatro o cinco púas. El trabajo está ilustrado con una lámina y doce figuras.

Translation by José F. Nonidez
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VARIATIONS IN THE CAROTID ARTERIES OF THE RABBIT

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ONE PLATE (TWELVE FIGURES)

That the blood-vessels of any group of mammals in general are subject to great variations is well known. Such minor variations as have been observed within any group have usually been ignored or at most, resolved to conform to the type. Using the rabbit as a basis of study in mammalian anatomy during the past two years, the writer has had an opportunity to make some interesting observations on the variations of the carotid arteries. Of one hundred and fourteen specimens dissected in the laboratory, twenty-three, or about 20 per cent, were found to differ from the usual condition described in the texts. Of these, eleven individuals possessed marked differences, as shown in the accompanying figures.

In the majority of cases, the common carotid artery (fig. 1, C.C.) passes forward from the superior thoracic aperture along the side of the trachea. Its branches include the superior thyroid artery supplying the thyroid gland, and the superior laryngeal artery. The latter arises at the level of the thyroid plate (larynx) and passes to the sternohyoid and sternothyroid muscles. A short distance cephalad the common carotid artery gives off a very small internal carotid artery which passes dorsad, and disappears beneath the auditory bulla. From this point forward the vessel is the external carotid artery, which gives off successively the occipital, the lingual, the external maxillary, the superficial temporal (one of the terminal branches), and the internal maxillary (the other terminal branch) arteries in the order named.

The occipital artery passes to the posterior portion of the head from the dorsal wall of the external carotid artery at a point just cephalad to the internal carotid.

The lingual artery arises from the ventral wall of the external carotid artery at a point about at the same level as the occipital and passes forward into the tongue.

The external maxillary artery is given off just cephalad of the lingual branch and passes to the medial surface of the ventral border of the mandible. It gives branches to the submaxillary gland and the muscles of mastication.

The internal maxillary and the superficial temporal arteries form the two terminal branches of the external carotid artery, the former passes in the direction of the orbit and gives off the inferior alveolar branch to the mandible, the latter passes to the temporal region and gives off the transverse facial artery to the cheek and face.

To simplify the presentation of differences found, it is convenient to use the following captions:

The internal carotid-occipital differences. A common difference in the relationships just noted is a condition where the internal carotid artery and the occipital branch arise from the common carotid as a single trunk, an innominate (figs. 2, 3, 7, and 12, *IN.*), which subsequently divides. Interesting gradations in respect to the division are found, from the condition (fig. 8) where the two arteries arise separately from the common carotid artery, and where there is no innominate formed, to that where a long innominate is formed as shown in figure 7. The order of the division is of interest also, since in some cases (figs. 2 and 3) the occipital branch is morphologically the most posterior, in others (figs. 7 and 12) the internal carotid artery occupies such a position. In the first condition there is no crossing of the two, the occipital passes dorsad to the muscles of the head and neck, and the internal carotid artery passes directly mesad under the auditory bulla. In the second condition there is a crossing, the occipital branch usually passing laterad of the deeper lying internal carotid trunk, although here again there seems to be some variability, since in one instance (fig. 4) the opposite is the case.

The external maxillary-lingual differences. While there has been noted no case where the sequential order varies in which these two arteries are given off from the external carotid artery, the relative differences in distances from one another in their origin is worthy of study. In two individuals (figs. 6 and 7) the interval between the two is very considerable, being nearly a centimeter apart. From this extreme, gradations occur, the distance between their points of origin on the carotid gradually approximating one another, as is shown in figures 3, 2, and 5, respectively. Finally, there is formed in some cases a common trunk, an innominate, before the division takes place, as shown in figures 4, 9, and 12. It is apparent that this approximation may take place in either direction; that is, the lingual may move cephalad to effect the junction with the maxillary, as shown in figure 9, or the maxillary may move caudad, as represented in figure 4. In the condition as shown in figure 5, both arteries have been slightly displaced from the usual position of either. In one case (fig. 6) it is interesting to note that the external maxillary is given off as a branch of the internal maxillary artery some distance cephalad of the latter's junction with the superficial temporal. In this case it occupies the relative position of the inferior alveolar branch, and might easily have been taken for the latter on superficial examination. The inferior alveolar branch in this case being somewhat more cephalad than usual. In two cases, however, figures 4 and 5, where the maxillary and lingual branches are closely approximated, the inferior alveolar branch is considerably more caudad than is ordinarily the case.

The internal maxillary-superficial temporal differences. In some instances these two vessels differ conspicuously in size, and where this condition is most marked, one may be considered a branch of the other. In conditions shown in figures 2 and 3 the superficial temporal is a small side branch passing dorsad, while the larger internal maxillary artery continues forward. In other cases (fig. 9) the opposite is true, the smaller internal maxillary artery is a branch of the larger superficial temporal trunk, and in this case its point of origin from the temporal is well cephalad.

In two cases represented by figure 7 the relationships of these terminal branches of the external carotid artery are of interest, since they together with the external maxillary artery form a three-parted fork; the external maxillary artery turns abruptly ventrad, the superficial temporal passes dorsad, and the internal maxillary bends mesad. In size there is very little difference between the three vessels, any one of which could be considered a terminal branch of the external carotid artery.

The superficial temporal-occipital differences. In three cases the occipital artery originates as a branch of the superficial temporal. In one individual (fig. 9) it passes dorsad from what may be considered the base of the superficial temporal or its innominate. In figure 5 it is but a little more cephalad, while in figure 10 it is shown passing away from the temporal well cephalad to the latter's junction with the other arteries.

Other differences. In one case shown in figure 11 all the arteries pass forward away from the common trunk in such a way as to form a sort of corona radiata. In such a condition the external carotid artery is practically eliminated, since the common carotid artery is broken up immediately into five terminal branches. In the condition shown in figure 12, the common carotid artery can be considered as terminating in three innominate trunks; one giving rise to the internal carotid and occipital branches, one forming the external maxillary-lingual branches, and the third, the internal maxillary-superficial temporal branches. In the condition shown in figure 10 the external carotid artery is very short, terminating in four branches, one of which is an innominate which forms the superficial temporal and occipital branches.

In two cases the inferior alveolar artery which normally is considered a branch of the internal maxillary, shows a tendency to branch well down on the external carotid trunk. This condition is indicated in figures 4 and 5. On the other hand, the external maxillary artery which normally is a branch of the external carotid, in two individuals (figs. 3 and 6), branches well cephalad from the internal maxillary artery.

SUMMARY

Variations in the relative positions and points of origin of the several vessels along the common carotid artery results in the formation of several innominate arteries. Those of especial interest are the occipital-internal carotid, the external maxillary-lingual, the internal maxillary-superficial temporal, and the superficial temporal-occipital arteries, represented in figures 2, 4, 9, and 10, respectively.

After the common carotid artery gives rise to the internal carotid or to the innominate (internal carotid-occipital), the remaining trunk, the external carotid artery, may terminate in a number of ways; it may end as a single trunk (either the internal maxillary or the superficial temporal); it may be bi-parted (as normally) or by two innominates, as in figures 9 and 12; it may be three-parted formed by the two maxillaries and the temporal, as in figure 7; it may be four-parted, formed by the two maxillaries, the temporal and the lingual, as in figure 8, or by the lingual, the two maxillaries and the innominate, as in figure 10; or it may be five-parted, formed by the two maxillaries, the lingual and the temporal and the occipital.

The inferior alveolar artery, which normally is a branch of the internal maxillary, is in two cases (figs. 4 and 5) well down on the external carotid artery.

The external maxillary artery in one case (fig. 6) is given off as a branch of the internal maxillary some distance cephalad to the latter's junction with the temporal. In several cases both the lingual and the external maxillary arteries pass out of the external carotid artery at the level of the internal maxillary and superficial temporal arteries, as in figures 8, 10, and 11.

The occipital artery varies considerably in its origin. It may be a branch from the internal carotid artery, as in figures 2, 3, 7, and 12; it may branch as an independent twig from the external carotid artery as in the normal condition, as shown in figures 4, 6, 8, and 11, or it may be a branch from the temporal, as in figures 5, 9, and 10.

In one case, figure 3, the lingual and the external maxillary arteries may be regarded as branches from the internal maxillary, since their points of origin are well cephalad to the point at which the temporal branch is given off.

PLATE 1

EXPLANATION OF FIGURES

1 Branches of the common carotid artery (left side) showing usual order and distribution of its various branches.

2 Variation in which the occipital and internal carotid arteries leave the common carotid artery together as an innominate artery. The superficial temporal is much smaller than the internal maxillary and can be regarded as a branch of the latter.

3 The innominate of the internal carotid and occipital, and the superficial temporal are close to each other, while the lingual, external maxillary, and inferior alveolar may be regarded as branches from the internal maxillary.

4 The external maxillary and lingual arteries arise as an innominate from the common carotid. The occipital and the internal carotid arteries are in the reverse sequence from the condition shown in figures 2 and 3, and the former passes mesad to the latter. The inferior alveolar is a branch from the external carotid.

5 The occipital leaves the superficial temporal close to the latter's base; the external maxillary and the lingual arteries arise close together from the external carotid, while the inferior alveolar is at the base of the internal maxillary.

6 The external carotid artery in this case terminates in three branches, the lingual, superficial temporal, and internal maxillary, the external maxillary and inferior alveolar being branches of the latter.

7 The external carotid artery terminates in three branches, the external maxillary, the internal maxillary, and the superficial temporal. Note the comparatively long innominate which divides to form the occipital and internal carotid arteries.

8 The lingual and external maxillary arteries originate close to the junction of the internal maxillary and superficial temporal arteries.

9 The external carotid terminates in two innominate arteries; one giving rise to the lingual and external maxillary arteries, the other forming the internal maxillary and superficial temporal arteries. The occipital branch is small and comes off at a point of junction of the two innominates.

10 The occipital artery is a branch of the superficial temporal which leaves the latter well cephalad.

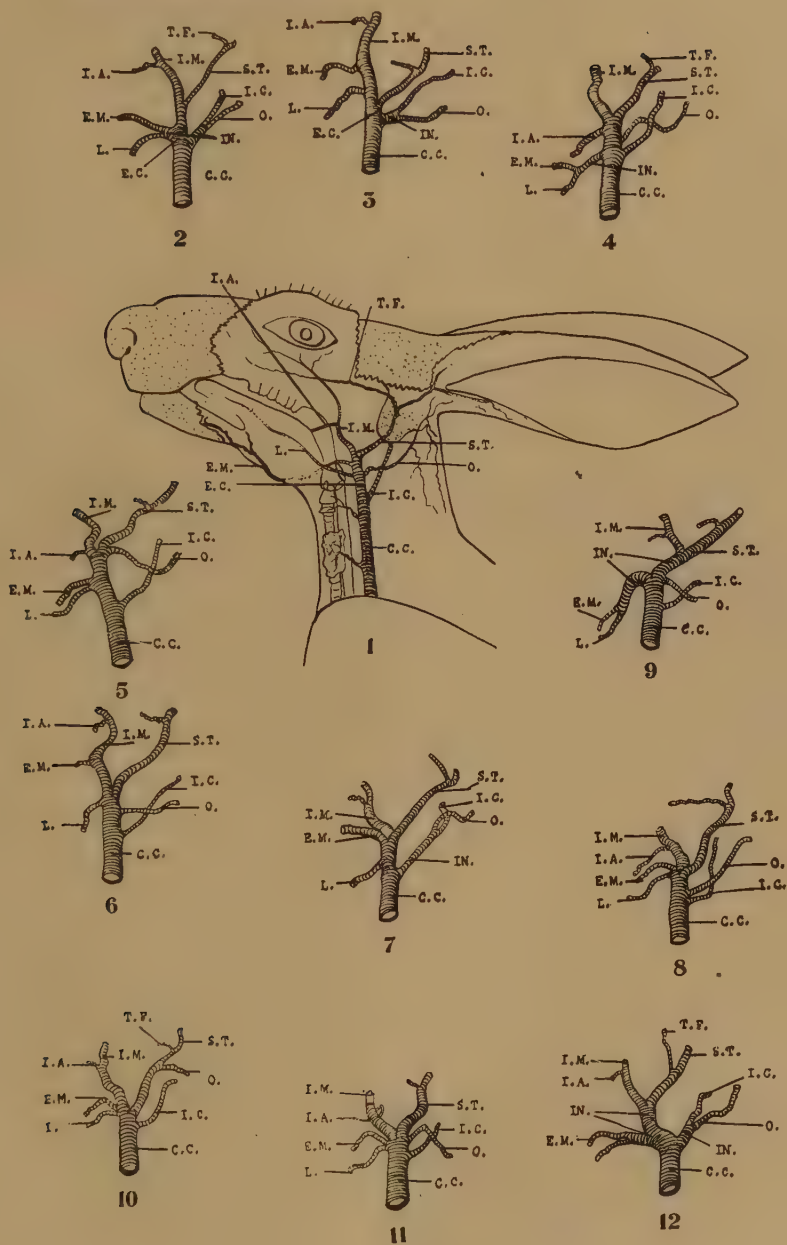
11 All the branches of the common carotid are close to one another, forming a sort of corona radiata as its termination.

12 The common carotid artery breaks in this case into three innominate arteries, the external maxillary-lingual, the internal maxillary-superficial temporal, and the internal carotid-occipital arteries.

ABBREVIATIONS

C.C., common carotid artery
E.C., external carotid artery
E.M., external maxillary artery
I.A., inferior alveolar artery
I.C., internal carotid artery
I.M., internal maxillary artery

IN., innominate artery
L., lingual artery
O., occipital artery
S.T., superficial temporal artery
T.F., transverse facial artery



Resumen por el autor, James Frederick Rogers.
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El pié como palanca.

Ha habido mucha discusión entre los anatómicos sobre la naturaleza de la palanca formada por el pié cuando el individuo se pone de puntillas. Algunos han considerado al pié como una palanca de primer grado, cuyo fulcro sería la articulación del tobillo, mientras que otros autores le comparan con una palanca de segundo grado, con el fulcro situado en el talón. La serie de palancas, peso y tensión de muelle ilustrados en el texto parecen probar de un modo indudable que la palanca es de primer grado cuando se usa el pié de este modo.

Translation by José F. Nonidez
Columbia University

THE LEVERAGE OF THE FOOT

JAMES FREDERICK ROGERS

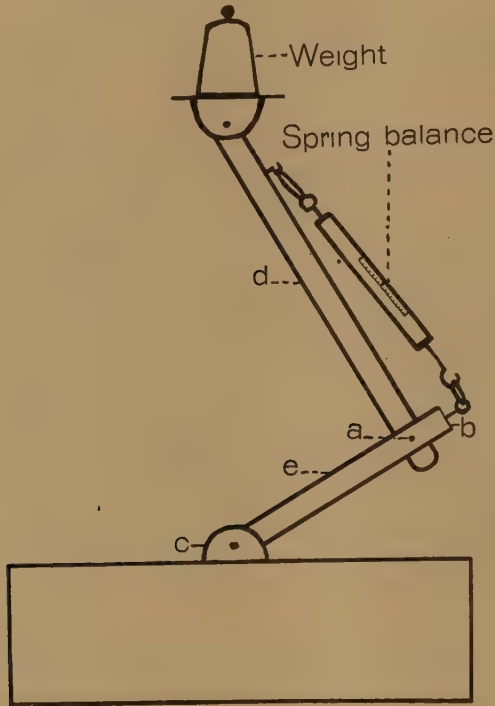
New Haven Normal School of Gymnastics, New Haven, Connecticut

ONE FIGURE

In most works on anatomy and physiology the bones of the foot working about the ankle-joint are, when the person owning the foot lifts himself on the ball, considered as behaving as a lever of the second class. Some authors state that there is a difference of opinion as to whether it becomes a lever of the second or of the first class, while a few assert positively that, no matter how used, this is always a lever of the first class, the fulcrum being at the ankle-joint. They explain that, when rising on the balls of the feet, we should consider ourselves, mechanically, as standing on our heads and pushing the earth from us with a force equal to the weight of the body. The new American edition of Gray makes this positive statement, but an excellent English work of about the same date of issue considers the lever in the more usual way.

Where there is so much antagonism of opinion, it would seem as if no one had taken the trouble to work out the problem experimentally, and we have never seen any suggestion of such an attempt. The device pictured herewith seems to solve the problem. Two pieces of lath, *d* and *e*, which represent the bones of the leg and foot, respectively, are hinged at *a*. The lower end of *e* (ball of foot) rests on a table directly or can be hinged in a block, *c*. A spring balance is attached in position to represent the calf muscles. A known weight, which represents the weight of the thigh and body above, is balanced on the upper end of *d*. If a weight of 5 pounds is used, a spring balance with a capacity of 25 pounds is most suitable. When the weight is in place and the machine held as in the drawing, the spring balance represent-

ing the pull of the calf muscles is read. This is always much greater than the amount of the weight and (with friction reduced to a minimum) in proportion to the relative length of the power and weight arms of a lever of the first class, the fulcrum being at *a*. Were this behaving as a lever of the second class, the power



arm would, of course, be longer than the weight arm, and the power needed to extend the foot would be less than the weight resting on the foot. Instead of using a fixed weight, *c* can be placed on a spring scale and pressure made downward upon the upper end of *d*. The reading of the scale will represent the weight of the body and that of the balance the pull of the muscles.

Resumen por el autor, Robert Wesley Henderson.

El sistema linfático del adulto de la ardilla terrestre rayada (*Spermophilus tridecemlineatus* Mitchell).

El presente estudio se llevó a cabo como preliminar de un estudio sobre el desarrollo del sistema linfático del embrión, y la presente descripción es lo mas próxima posible a la de la forma tipo de sistema linfático en este animal, incluyéndose en ella la disposición anatómica, posición y relación de los vasos válvulas, nódulos y sacos linfáticos, áreas de drenaje y orificios veno-linfáticos. Los puntos especiales de mas interés son las descripciones de los orificios linfáticos renales y post-cavos, la de un saco-linfático yugulo-subclavio en el adulto y la de una fórmula para la inyección de una masa especial ideada para vencer los obstáculos inesperados encontrados en este animal, siendo la mayor dificultad para conseguir buenos preparados el extraordinario poder decolorante de los fluidos de los tejidos sobre el azul de Prusia. La nomenclatura empleada se aproxima lo mas posible a la usada en la anatomia humana. La relación y variación de estructura están ilustradas por seis figuras.

Translation by José F. Nonidez
Columbia University

THE ADULT LYMPHATIC SYSTEM OF THE STRIPED GROUND-SQUIRREL (*SPERMOPHILUS TRI- DECEMILINEATUS* MITCHILL)

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SIX FIGURES

The original purpose of this study of the gross adult anatomy of the lymphatic system of the striped ground-squirrel (*Spermophilus tridecemlineatus*) was that it should be merely a preliminary to a study of the development of the lymphatic system in the embryo. However, so much difficulty was encountered in the securing of an injection mass which would give good differentiation and yet remain permanent that the work practically resolved itself into a search for an injection mass which would fulfill these requirements in this species of animal.* When this mass was finally made, the work of dissecting out and describing the adult lymphatic system was a very simple matter. No work has as yet been done on the embryology.

Forty-eight specimens were successfully injected in some region. So this description is as near as possible that of the type form of lymphatic system in this animal, including the more unusual variations.

India-ink gelatin mass was used as the injecting media. Formula for making:

1. Finely powder stick India ink in a mortar.
2. Add 4 to 5 cc. saturate solution NH_4OH to the powder in the mortar. Stir with pestle till thick and syrupy.
3. Allow to stand till coarse particles of ink settle.
4. Decant.

*Berlin blue gelatin mass exactly filled all the requirements of an injection mass except that the tissue fluid of this animal would completely decolorize it in a few minutes.

5. Soften plate gelatin in distilled water and liquefy over warm water-bath, using no more water than absolutely necessary.

6. Thoroughly stir India-ink-ammonia solution into gelatin.

7. Strain through several layers of cheese-cloth and allow to stand in warm place for several days.

8. As a preservative add a small crystal each of thymol and potassium iodide.

9. Keep in tightly stoppered bottle.

The injecting apparatus consisted of a 5-cc. glass syringe and a steel hypodermic needle.

Ether, chloroform, and illuminating gas were the killing agents. The best results, however, were obtained from those specimens killed with illuminating gas.

Injections were made in most specimens while the animal was still warm and as soon as possible after death, though good injections were secured from nodes after the animal had been several days dead and preserved in a 5 per cent formalin solution. The best cutaneous injections were obtained in females heavy with milk.

Cutaneous injections were made in the soles of the feet, between the toes, in the heels, in the back, at the base of the tail, in the lips of the rectum, vulva and mouth, in the sides and abdomen, at the bases of the ears, and the tip of the nose. Injections were also made in the tip of the tongue, walls of the intestines, the stomach, the testes, in the spleen, the anterior inguinal nodes, the intestinal nodes, the cervical and axillary nodes, and one injection was made in the right thoracic node.

In making cutaneous injections the best results were obtained from superficialsubcutaneous injections, since the mass spread out from the point of injection as a fine anastomosing network which finally formed one or more large capillaries. Injections in the superficial fascia merely formed large lakes, as a rule, except in the feet and the head where the lymph systems were nearly always injected.

The intestinal lymphatics were easily demonstrated by starving the animal for a day or so and then feeding it suet. If the animal was killed two or three hours after such feeding the intes-

tinal lymphatics would be completely demonstrated to the minutest detail by the presence of chyle in the intestinal walls and the mesenteries. The intestinal lymphatics may also be demonstrated by injection if care is taken not to break the mucosa.

Not a single successful injection of the spleen and the walls of the stomach was made, though it was tried repeatedly.

Injection of the lymph nodes was nearly always successful if the point of the needle was completely included in the node.

LYMPHATIC SYSTEM

The lymphatic system of the common ground-squirrel, exclusive of the digestive tract, is essentially bilaterally symmetrical and consists of three elements, lymph vessels, lymph nodes, and lymph sacs.

The lymph vessels are transparent, thin-walled tubes of very small caliber and lie in the connective tissue surrounding blood-vessels, in the skin, and in some muscles. The thoracic duct, which is the largest vessel in the lymphatic system, when distended with an injection mass has an average diameter of less than 1 mm. Many other lymph vessels even when filled with a colored injection mass can be detected only with the aid of a microscope. These vessels transfer the lymph either from tissues to nodes, from node to node, from nodes to sacs, or from nodes or sacs to empty it into blood vessels. Their arrangement will be described in detail, with the description of the nodes, as afferent vessels, those carrying lymph to the nodes, and efferent vessels, those carrying lymph away from the nodes. Some vessels, since they carry lymph from one node or group of nodes to another, will be described under both heads.

The lymph nodes will be denominated according to the location in, or near, which they lie and as nearly as possible in accordance with the nomenclature used in human anatomy. Not all nodes, however, will be given individual names, as certain nodes vary greatly in their frequency of appearance and location, so, for the sake of simplicity, they will be treated in groups whenever convenient. According to location, the nodes may be divided into the cervical, axillary, thoracic, appendicular, inguinal, lumbar, cisternal, and intestinal groups.

CERVICAL NODES AND VESSELS

This group, which will be treated as superficial cervical and deep cervical nodes, is difficult to identify from the other glands of the neck unless it is injected.

The superficial cervical nodes lie along the course of the external jugular vein and its branches, and are usually four in number, the anterior parotid node, the posterior parotid node, the superficial thyroid node, and the submaxillary node.

The anterior parotid node (fig. 1, *Ant.Par.N.*) lies on the anterior surface of the parotid gland at the junction of the anterior auricular and posterior facial veins. Its afferents come from the anterior portion of the ear and the dorsal part of the head, and its efferents pass posteriorly along the posterior facial vein to the posterior parotid node, or to the level of the internal carotid artery along which they may pass directly mesial to the common carotid and then posteriorly to the deep cervical nodes. Size: approximately, $0.5 \times 0.5 \times 0.5$ mm.

The posterior parotid node (fig. 1, *P.Par.N.*) is located on the posterior surface of the parotid gland near the junction of the posterior auricular and external jugular veins. Afferent vessels come from the posterior portion of the ear and the dorsal side of the head and neck. The efferent vessels may pass ventrally and posteriorly along the external jugular vein to the superficial thyroid node or may go forward along the posterior facial vein to pass directly to the deep cervical nodes along with those from the anterior parotid node. Size: $3 \times 2 \times 1$ mm.

The superficial thyroid node (fig. 1, *Sup.Thy.N.*) is located under the platysma on the lateral surface of, and immediately in contact with, the thyroid gland in the angle formed by the junction of the anterior facial vein and the external jugular vein. It receives afferent vessels from the skin of the chest, which pass anteriorly over the external jugular vein, and usually a vessel from the posterior and anterior parotid lymph nodes. Size: approximately, $2.5 \times 1.5 \times 1$ mm.

The submaxillary nodes (fig. 1, *Sub.Max.N.*) usually two in number, are located at the anterior margin of the submaxillary

gland on either side of the anterior facial vein, at the junction of the internal and external maxillary veins. They are just ventral to the tendon of the digastric muscle, lateral to the sternothyroid muscle and covered by the platysma and skin. They receive afferent vessels from the lower lip which parallel the external maxillary vein or pass over the surface of the anterior belly of the digastric muscle; from the upper lip which parallel the angular vein; from the tongue which parallel the internal maxillary vein or pass directly through the musculature of the tongue, and from the lateral thyroid node along the anterior facial vein. They send efferent vessels to the lymphatic plexus surrounding the larynx. This plexus connects with the corresponding nodes of the opposite side. They also send vessels which pass over the internal maxillary vein mesially to a point near the external carotid artery and then follow posteriorly along the external carotid and common carotid arteries to the deep cervical nodes. Size: approximately, $2.5 \times 2.5 \times 0.5$ mm.

The deep cervical nodes (fig. 1, (*D.Cer.N.*)) are usually two in number and are located in the angle between the sternocleidomastoid and sternohyoid muscles, ventral to the common carotid artery and the internal jugular vein, and lateral to the trachea. One of these nodes is usually considerably larger than the other. They receive afferent vessels directly from the tongue (fig. 1, *T.*), from the lymphatic plexus over the larynx, from the submaxillary lymph nodes, and sometimes from the anterior and posterior parotid nodes. In fact, all the lymph from the superficial cervical nodes and other lymphatics of the head and neck eventually passes through these nodes.

The efferent vessels, usually two in number, pass posteriorly, the larger along the common carotid artery and the other along the internal jugular vein. On the right side, very close to the junction of the internal and external jugular veins, these lymph vessels unite and enter the veins near the jugulosubclavian junctions. Size: $4 \times 3 \times 2$ mm. and $2 \times 1 \times 0.5$ mm.

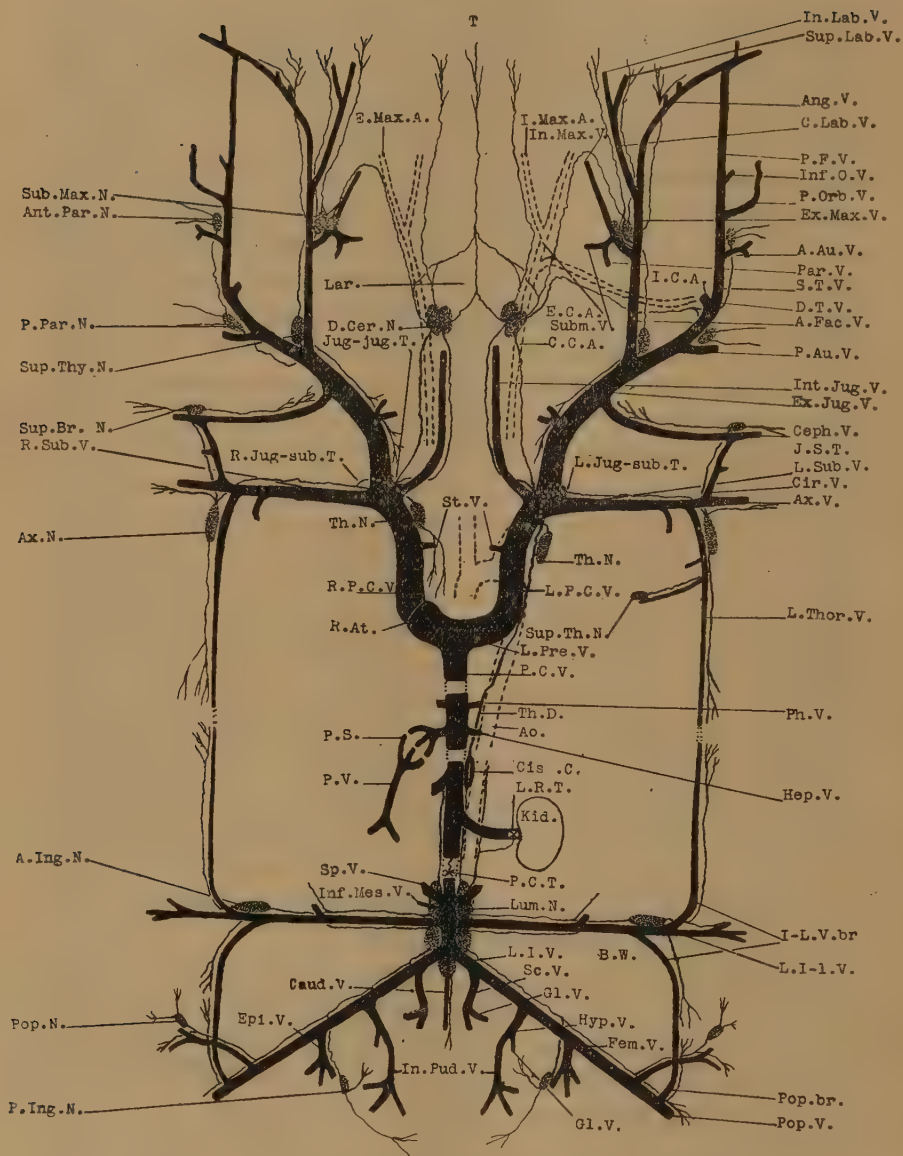


Fig. 1 Venous and lymphatic systems (diagrammatic)

ABBREVIATIONS

<i>ao.</i> , aorta	<i>L.Sub.V.</i> , left subclavian vein
<i>Ang.V.</i> , angular vein	<i>Lum.N.</i> , lumbar node
<i>A.Au.V.</i> , anterior auricular vein	<i>L.R.V.</i> , left renal vein
<i>Ant.Fac.V.</i> , anterior facial vein	<i>L.I.V.</i> , left iliac vein
<i>Ant.Par.N.</i> , anterior parotid node	<i>L.I.-I.V.</i> , left ilio-lumbar vein
<i>A.Ing.N.</i> , anterior inguinal nodes	<i>L.E.Jug.V.</i> , left external jugular vein
<i>Ax.N.</i> , axillary node	<i>L.P.C.V.</i> , left precava
<i>Ax.V.</i> , axillary vein	<i>L.R.T.</i> , left renal tap
<i>In.A.</i> , innominate artery	<i>L.Thor.V.</i> , long thoracic vein
<i>B.W.</i> , body wall	<i>L.Jug.-sub.T.</i> , left jugulo-subclavian tap
<i>B.I.-L.V.</i> , branch of iliolumbar vein	<i>Lar.</i> , larynx
<i>Cis.C.</i> , cisterna chyli	<i>P.V.</i> , portal vein
<i>Ceph.V.</i> , cephalic vein	<i>P.C.V.</i> , postcava
<i>Cir.V.</i> , circumflex vein	<i>P.S.</i> , portal system
<i>Caud.V.</i> , caudal vein	<i>P.Orb.V.</i> , postorbital vein
<i>Com.Lab.V.</i> , common labial vein	<i>Pop.V.</i> , popliteal vein
<i>C.C.A.</i> , common carotid artery	<i>Pop.B.</i> , popliteal branch
<i>C.A.</i> , celiac axis	<i>P.Au.V.</i> , posterior auricular vein.
<i>D.T.V.</i> , deep temporal vein	<i>Par.V.</i> , parotid vein
<i>D.Thor.V.</i> , dorsal thoracic vein	<i>P.Co.A.</i> , posterior colic artery
<i>D.Cerv.N.</i> , deep cervical nodes	<i>P.Par.N.</i> , posterior parotid node
<i>Ex.Jug.V.</i> , external jugular vein.	<i>Post.T.</i> , postcaval tap
<i>E.C.A.</i> , external carotid artery	<i>P.Ing.N.</i> , posterior inguinal node
<i>E.Max.A.</i> , external maxillary artery	<i>P.F.V.</i> , posterior facial vein
<i>Epi.V.</i> , epigastric vein	<i>Ph.V.</i> , phrenic vein
<i>E.Max.V.</i> , external maxillary vein	<i>R.P.C.V.</i> , right precava
<i>Fem.V.</i> , femoral vein	<i>R.Jug.-sub.T.</i> , right jugulosubclavian tap
<i>Gl.V.</i> , gluteal vein	<i>R.Sub.V.</i> , right subclavian vein
<i>Hep.V.</i> , hepatic vein	<i>St.V.</i> , sternal vein
<i>Int.V.</i> , intercostal vein	<i>Sc.V.</i> , sciatic vein
<i>Inf.O.V.</i> , inferior orbital vein	<i>Sp.V.</i> , spermatic vein
<i>Hyp.V.</i> , hypogastric vein	<i>Sub.Max.N.</i> , submaxillary nodes
<i>In.Pud.V.</i> , internal pudic vein	<i>Subm.V.</i> , submaxillary vein
<i>Int.Jug.V.</i> , internal jugular vein	<i>Sup.Thy.N.</i> , superficial thyroid node
<i>I.C.A.</i> , internal carotid artery	<i>S.T.V.</i> , superficial temporal vein
<i>Inf.Lab.V.</i> , inferior labial vein	<i>Sup.Br.N.</i> , superficial brachial node
<i>In.Max.A.</i> , internal maxillary artery	<i>Sup.Th.N.</i> , superficial thoracic node
<i>In.Pud.V.</i> , internal pudic vein	<i>Sup.Lab.V.</i> , superior labial vein
<i>In.Max.V.</i> , internal maxillary vein	<i>Th.D.</i> , thoracic duct
<i>Inf.Mes.V.</i> , inferior mesenteric vein	<i>Th.N.</i> , thoracic node
<i>Jug-jug.T.</i> , jugulojugular tap	
<i>Kid.</i> , kidney	

AXILLARY NODES AND VESSELS

In the axilla there is usually found but a single large axillary node (fig. 1, *Ax.N.*), though occasionally a small node is also present. These nodes lie close to the long thoracic vein immediately posterior to its junction with the axillary vessels. It is embedded in the anterior lateral side of a large gland. It receives afferent vessels from the skin of the thorax which pass to it anteriorly along the branches of the long thoracic vein; from a small node, the superficial thoracic node (fig. 1, *Sup.Th.N.*), which lies just behind the shoulder close to a branch of the long thoracic (this node either is not always present or else cannot always be found); from the front leg and the sole of the foot, which vessels parallel the brachial artery and its branches; from the skin over the side of the front leg, which vessels pass obliquely across the musculature at about the middle of the humerus to terminate at the anterior end of the axillary gland; from the superficial brachial node, which vessels pass through the musculature of the leg with the circumflex vein.

The efferent vessels extend from the anterior end of the axillary node along the subclavian vein and artery to empty, on the right side, into the veins between the external jugular and subclavian veins at their junction. On the left side it unites with the thoracic duct or the jugular lymph sac if present. Size: approximately, 7 x 2 x 1 mm.

THORACIC NODES AND VESSELS

The thoracic nodes (fig. 1, *Th.N.*), are two in number, one on each side, and lie in the fatty tissue near the walls of blood-vessels. The node on the left side is located posterolateral to, and on the wall of, the subclavian artery at its anterior end. It is a rather large node, measuring 6 x 2 x 1 mm. Its afferent vessels have not been determined, but its efferent vessel is short and empties into the thoracic duct or into the efferent from the left axillary node near its termination.

The right thoracic node is somewhat smaller and is located more ventrally along the mesial wall of the right precava near its

anterior end. Afferent vessels come from the thymus, the anterior walls of the trachea, and the region of the heart and lungs. The distribution of these vessels has not been exactly determined, as only one specimen has yielded to injection and that only partially. The afferent vessels pass anteriorly along the precava and around it dorsally to unite with the efferent vessel from the right axillary node just before it makes connection with the veins.

APPENDICULAR NODES AND VESSELS

The superficial brachial node (fig. 1, *Sup.Br.N.*) lies subcutaneously between the triceps and deltoid muscles posterior to the cephalic vein at its junction with the circumflex vein. It receives afferent vessels from the dorsal side of the foot and foreleg and from the skin, and also vessels from the shoulder which parallel the upper portion of the cephalic vein. Its efferents lead to the axillary node over the circumflex vein. Size: approximately $2 \times 2 \times 0.5$ mm.

The popliteal node (fig. 1, *Pop.N.*) lies back of the knee in the hind leg, in the popliteal space, and is buried in fat. It is a small gland approximately $2 \times 1.5 \times 0.5$ mm. It receives afferent vessels from the sole of the foot and the skin which pass proximally through the skin on the posterior surface of the gastrocnemius to a point opposite the popliteal node. Then they pass between the biceps femoris and semimembranosus muscles in the fat to the lymph gland. It also receives afferent vessels from the mesial and posterior surface of the leg and from the region around the anus.

The efferent vessels pass proximally along a branch of the popliteal vein and the popliteal vein to the femoral vein where it unites with other lymph vessels.

INGUINAL NODES AND VESSELS

The inguinal nodes may be divided into anterior inguinal nodes (figs. 1 and 4, *A.Ing.N.*) and the posterior inguinal nodes (fig. 1, *P.Ing.N.*).

The posterior inguinal nodes are single, small, approximately $2 \times 1.5 \times 1$ mm., and are very difficult to find unless they are in-

jected as they lie buried in the superficial fascia on the posterior abdominal wall, just lateral to the ventral midline and along a branch of the epigastric artery. Afferent vessels come from the mesial side of the hind leg, the region of the tail and anus, and the posterior abdominal wall. Efferent vessels pass along the branch of the epigastric, near which the node lies, to the epigastric and iliac veins, where they unite with other lymphatic vessels from the leg.

The anterior inguinal nodes may be single or number as many as five. If single, they are large, approximately $7 \times 2 \times 2$ mm., and if more than one they may be as small as $2 \times 1 \times 1$ mm. The usual number is two or three with one more node on the left side than on the right. These nodes lie along the iliolumbar vein and receive afferents from the skin over the front and lateral side of the hind leg even as far posterior as the tail and anus, and from the skin over the sides and ventral abdominal wall. Their efferents pass ventrally along the iliolumbar vein to the abdominal wall where they join lymph vessels from the musculature of the abdominal walls.

LUMBAR NODES AND VESSELS

The lumbar nodes (fig. 1, *Lum.N.*), are arranged around the posterior end of the aorta where the spermatic (ovarian), iliolumbar, inferior mesenteric, and iliac arteries are given off. The simple type form of their arrangement is a group of five nodes; one on each side of the aorta just anterior to the iliolumbar arteries; one on each side of the aorta just posterior to the iliolumbar arteries and just anterior to the right and left iliac arteries, respectively, and one caudal node, which lies between the iliac arteries at the bifurcation of the aorta. It is very seldom, however, that this simple type occurs, it being found only once in all the specimens dissected, and in that case the nodes on the right of the aorta were so closely connected across the ventral side of the right iliolumbar artery and vein that it was doubtful whether they were in the same or in separate capsules. If, however, the nodes lateral to the aorta are completely separate anteroposteriorly, the caudal node varies and has been found to con-

sist of as many as two single nodes and a double node. In other specimens the caudal node has been found to be entirely lacking, in which case the other nodes are of larger size. In the majority of specimens the caudal node is present and single, and, on one side or the other, one pair of the lateral lumbar nodes are united with each other anteroposteriorly across the iliolumbar artery and vein on the ventral side to form a single or a double node. The size of these nodes is extremely variable, 14 mm. being the length of the longest node found, and 2.5 mm. the greatest width, while the smallest node was approximately 1 x 1 x 1 mm. These nodes are all connected by a very rich plexus of lymph vessels, so that if an injection from any point fills any of the nodes it will usually fill all. Occasionally, however, injections from the anterior inguinal nodes would fail to inject the caudal node, which result was never the case if the injection was made from the hind feet, tail, or posterior inguinal nodes.

The afferent vessels to these nodes are very numerous since they drain the whole posterior region of the body. Several vessels come from the hind legs along the iliac veins and receive branches accompanying the saphenous vein from the dorsum of the hindfoot and branches from the popliteal node and the posterior inguinal node. A lymph vessel comes from the tail accompanying the caudal vein and artery. From the anterior inguinal nodes usually two vessels parallel the iliolumbar artery and vein to the body wall, where a junction is made with other lymph vessels coming from the musculature of the body wall over branches of the iliolumbar blood-vessels. From the body wall to the lumbar nodes the lymph channels vary from one single vessel to as many as three, in which case a richly anastomosing plexus of vessels is formed surrounding the iliolumbar vein and artery. A lymph vessel comes from each testis along the spermatic vein and artery and another passes through the mesentery from each testis to follow a branch of the hypogastric vein, hypogastric and iliac veins to the lumbar nodes. Still another lymph vessel follows the inferior mesenteric artery and vein from the large intestine to the lumbar nodes.

Of the efferent vessels from the lumbar nodes only one is constant and lies between the aorta and the postcava. A second which lies on the left wall of the aorta is constant in the most of the specimens, though it sometimes passes over the ventral side of the aorta to unite with the more constant vessel. A third is occasionally present which passes from the lumbar nodes along the right side of the postcava. These vessels may vary from the almost simple condition as described to a very rich lymph plexus continuous from the lumbar nodes to the cisternal nodes and cisterna chyli over the surface of the aorta and sometimes over both the aorta and the postcaval vein.

CISTERNAL NODES AND VESSELS

The cisternal nodes (figs. 3, 4, 5, and 6, *Cis.gr.*) are small nodes not larger than $5 \times 1.5 \times 0.5$ mm. and vary in number from one to three. One, which is located just anterior to the left renal artery (figs. 3, 4, 5, and 6), is always present and always in this position. Of the others any or all may be present or all may be absent, and when present are exceedingly variable in position. One may be found posterior to the left renal artery (fig. 5), another posterior to the right renal artery (fig. 5) or anterior to the right renal artery (fig. 4), in which case there are sometimes two nodes present. The only afferent vessels of these nodes which have been demonstrated are those coming from the lumbar nodes, and their efferents all lead to the cisterna chyli.

INTESTINAL NODES AND VESSELS

The intestinal lymphatics comprise the superior mesenteric, gastric, and inferior mesenteric groups of nodes and their allied lymph vessels.

The superior mesenteric nodes (fig. 2, *Sup.Mes.N.*) are a large group of nodes near the cecum, lying mainly on the left side of the mesenteric veins and arteries. These nodes are subject to great variation and may consist of a large number of small nodes closely grouped together or may consist of what appears to be a single large node having several parts, usually three or four. In any case the most distal end of the group lies along the cecal branch

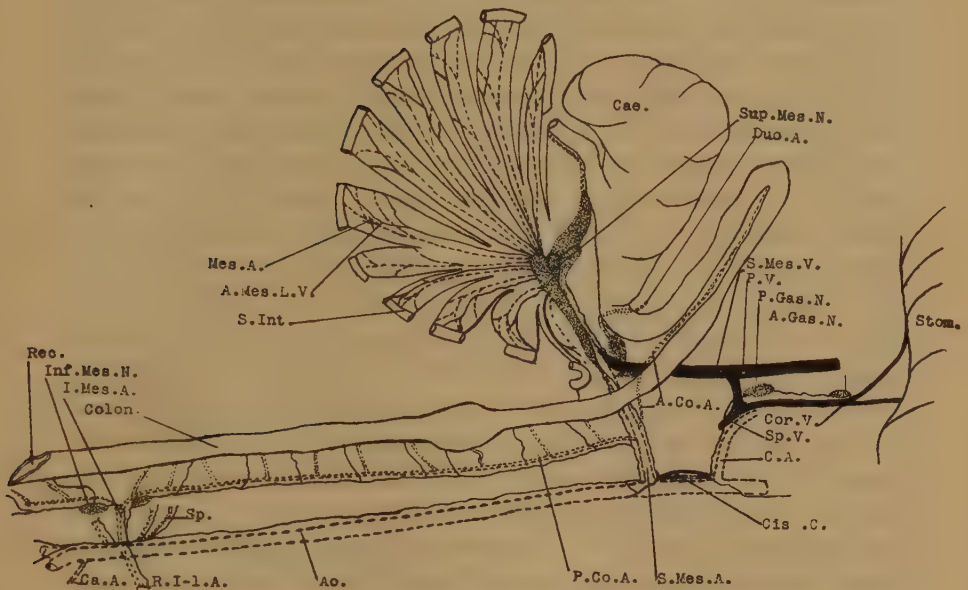


Fig. 2 Lymphatics of the digestive tract (diagrammatic)

Ao., aorta
 A.Mes.L.V., afferent mesenteric lymph vessel
 A.Gas.N., anterior gastric node
 A.Co.A., anterior colic artery
 Colon,
 C.A., celiac axis
 Cor.V., coronary vein
 Cis.C., cisterna chyli
 Cae., caecum
 Ca.A., caudal artery
 Duo.A., duodenal artery
 Inf.Mes.N., inferior mesenteric node
 I.Mes.A., inferior mesenteric artery

Mes.A., mesenteric artery
 P.V., portal vein
 P.Gas.N., posterior gastric node
 P.C.A., posterior colic artery
 R.I-l.A., right ilio-lumbar artery
 Rec., rectum
 Sp., spermatic artery
 S.Int., small intestine
 Sup.Mes.N., superior mesenteric node
 S.Mes.A., superior mesenteric artery
 S.Mes.V., superior mesenteric vein
 Stom., stomach
 Sp.V., splenic vein

of the superior mesenteric artery and vein, its distal end in contact with the cecum at the junction of the cecum and small intestine. At the junction of the cecal blood-vessels with the rest of the mesenteric blood-vessels this portion of the node is continuous with a greatly expanded portion of the group which overlies all of the blood-vessels and continues as a gradually diminishing node to approximately a point where the superior mesenteric artery and vein become parallel with each other in the mesentery. This latter portion may consist of several small nodes which lie along the mesenteric blood-vessels at their proximal terminations, but which are more or less continuous with the main portion of the group. At the proximal end of the group, between the angle formed by the divergence of the superior mesenteric vein and artery from their parallel position in the mesentery, is frequently a small node, approximately $3 \times 1 \times 0.5$ mm. Almost continuous with it and lying along the mesenteric blood-vessels to the posterior end of the duodenum is a slightly larger node, $5 \times 2 \times 1$ mm., which is always present.

The afferent vessels to the superior mesenteric group of nodes pass through the mesentery from the walls of the small intestine, in general parallel with the mesenteric blood-vessels, though not necessarily close to them. In the walls of the intestine and cecum these afferent mesenteric lymph vessels are continuous with very fine plexuses of lymph vessels. However, there has never been any evidence of such plexuses in the mesentery. Apparently the afferent mesenteric lymph vessels, in the mesentery, are merely conducting vessels and not collecting vessels.

The efferent mesenteric lymph vessels pass, usually as a plexus, to the cisterna chyli over the surface of the superior mesenteric artery.

Anterior to the superior mesenteric group of nodes is a group of two nodes which, from their position, have been designated the gastric nodes, anterior and posterior, respectively.

The posterior gastric node (fig. 2, *P. Gas. N.*) is approximately $4 \times 2 \times 1$ mm. in size and lies in the mesentery near the celiac axis in the angle formed by the splenic and coronary veins. A short distance anterior to the posterior gastric node, on the wall of the

coronary vein and near the cardiac wall of the stomach, is the anterior gastric node (fig. 2, *A. Gas. N.*) which is sometimes larger and sometimes smaller than the posterior gastric node.

The efferent vessels from the posterior gastric node pass over the surface of the celiac axis to the cisterna chyli and those from the anterior gastric node pass along the coronary vein to the posterior gastric node or to its efferents. The afferent vessels of the anterior gastric node have never been demonstrated, but from its position it is inferred that they come from the stomach.

The inferior mesenteric nodes (fig. 2, *Inf. Mes. N.*) when present, are usually two in number and lie in the mesentery on either side of the bifurcation of the inferior mesenteric artery and vein dorsal to the colon. The larger is usually $2 \times 1 \times 0.5$ mm. in size, and the other, when present, is about half as big. The larger is the most posterior and receives an afferent vessel along the dorsal side of the rectum which closely parallels the rectum and receives branches along its course from the rectal walls. The more anterior node receives an afferent vessel which parallels the anterior branch of the inferior mesenteric artery and may connect anteriorly with a small node, sometimes present, in the mesentery near the superior mesenteric nodes which connects with the superior mesenteric nodes. All along its course it receives branches from the intestine.

The efferent vessels from the inferior mesenteric nodes pass dorsally on either side of the inferior mesenteric artery for a short distance, when they unite to form one vessel which enters the lumbar group.

In some specimens the inferior mesenteric nodes are not present, in which case the lymph from the rectum and posterior colon drains into the lumbar nodes through several channels which pass dorsally in the mesentery.

CISTERNA CHYLI

The cisterna chyli (figs. 1, 3, 4, 5, and 6, *Cis. C.*) is the only lymph sac which may be considered as constantly present in the ground-squirrel and is a sacculation of the lymph vessels which lie, in the greatest number of cases, on the ventral and lateral

sides of the aorta between the superior mesenteric artery and the celiac axis. It is a rather variable structure and may be merely a rich plexus of lymph vessels or it may be a well-defined sac almost completely surrounding the aorta, or it may be divided into two sacs, one in the usual location and the other a little forward on the dorsal surface of the aorta. Its afferent vessels are the vessels from the cisterna, lumbar, and intestinal nodes and its efferent is the thoracic duct.

THORACIC DUCT

The thoracic duct (figs. 1, 2, 3, 4, 5, and 6, *Th.D.*) is the largest lymph duct in the body and extends from the cisterna chyli along the right dorsolateral side of the aorta forward to the aortic arch and then along the dorsal side of the left subclavian artery anterior to the first rib, where it turns ventrally to tap the venous system at the junction of the external jugular and left subclavian veins.

The thoracic duct is not always a simple vessel (fig. 5, *R. and L.Th.D.*) in fact, in most of the cases the posterior half is a more or less complex plexus of lymphatic vessels and in some cases a distinct left thoracic duct is evident. In one case (fig. 6, *L.Th.D.*)

ABBREVIATIONS

<i>Ao.</i> , aorta	<i>L.I.V.</i> , left iliac vein
<i>Az.V.</i> , azygos vein	<i>L.I-l.A.</i> , left iliolumbar artery
<i>A.Ing.N.</i> , anterior inguinal node	<i>L.I-l.V.</i> , left iliolumbar vein
<i>B.C.A.</i> , brachiocephalic artery	<i>L.Jug.Sub.T.</i> , left jugular-subclavian tap
<i>B.W.</i> , body wall	<i>L.Th.D.</i> , left thoracic duct
<i>Clav.</i> , clavicle	<i>L.E.Jug.V.</i> , left external jugular vein
<i>Cis.C.</i> , cisterna chyli	<i>L.P.C.V.</i> , left precaval vein
<i>C.A.</i> , coeliac axis	<i>P.C.V.</i> , postcaval vein
<i>Car.A.</i> , carotid arteries	<i>R.I-l.V.</i> , right iliolumbar vein
<i>Dia.</i> , diaphragm	<i>R.I-l.A.</i> , right iliolumbar artery
<i>I.Mes.A.</i> , inferior mesenteric arteries	<i>R.Th.D.</i> , right thoracic duct
<i>Kid.</i> , kidney	<i>S.Mes.A.</i> , superior mesenteric artery
<i>L.R.V.</i> , left renal vein	<i>Sac.</i> , sacculatation
<i>L.Sub.A.</i> , left subclavian artery	<i>Sp.</i> , spermatic (ovarian) arteries
<i>L.I.A.</i> , left iliac artery	<i>S.Int.A.</i> , superior intercostal artery
<i>L.Sub.V.</i> , left subclavian vein	

the left thoracic duct, instead of passing dorsally around the aorta to unite with the thoracic duct proper, as is usually its termination, extended forward to a point opposite the seventh rib, then swung to the left and extended diagonally lateral and forward to the posterior end of the left superior intercostal artery, where it could be traced no farther.

VENO-LYMPHATIC TAPS

The exact location at which the lymphatics tap the venous system is constant for only two points. On the right side the efferent lymph vessels from the axillary node tap the veins in, and a little to the dorsal side of, the angle formed by the junction of the right external jugular and right subclavian veins (fig. 1, *R.Jug.-sub. T.*), while the efferent vessels from the deep cervical nodes make a similar tap in the angle formed by the junction of the internal and external jugular veins (fig. 1, *Jug.-jug.T.*).

A third tap which is constant in most specimens is the jugulo-subclavian tap (figs. 1, 3, 4, and 5, *L.Jug.-sub.T.*) on the left side. This tap is located in most cases at the same relative point as that on the right side and usually receives the lymph from the thoracic duct and the efferents from the left deep cervical and the left axillary nodes, which vessels unite, just at the point of junction, with the veins. Some rather interesting exceptions, however, have been found.

In one specimen the lymph vessels from the left deep cervical nodes connected with the veins in the angle formed by the left internal and external jugular veins, while the thoracic duct and efferent vessels from the left axillary region made the usual tap in the jugulosubclavian junction.

In another specimen the efferent vessel from the left deep cervical nodes, which follows the internal jugular vein, tapped the veins in the jugulojugular junction, while the efferent vessel from the left deep cervicals which follows the common carotid artery made the usual jugulosubclavian junction with the thoracic duct and left axillary efferent vessel.

In two other specimens the thoracic duct, left deep cervical vessels and the left axillary vessels united to form a small but

perfectly distinct lymph sac dorsal to the left external jugular vein at its posterior end. From this lymph sac a short lymph vessel passed ventral to tap the left external jugular vein on its dorsal surface.

Two other taps were found in the posterior end of the body which are unusual, at least direct evidence of their presence is unusual. Each tap was demonstrated but once and both occurred in the same specimen. One was in the postcava (fig. 1, *P.C.T.*) and the other was in the left renal vein (fig. 1, *L.R.T.*).

The injection which demonstrated the presence of the renal and postcaval tap was made in the right inguinal node. The mass was seen to pass into the lumbar nodes and start forward toward the cisternal nodes. At almost the same instant the mass appeared in the postcava. As the mass filled the lumbar efferent vessels and cisternal nodes, it passed through a lymph vessel, on the posterior side of the left renal vein, in a diagonal direction, lateral and anterior, over the lumbar musculature. At a point near the left renal vein the mass disappeared under some fatty tissue, and almost simultaneously near the hilus of the kidney was seen to appear in the renal vein.

Later an attempt was made to prove the presence of these taps by dissection in the following manner. The animal was hardened in formalin solution and the veins opened longitudinally on the ventral side. The hardened blood was then removed piece by piece, the work being done with needles under a high-power binocular microscope. After the blood was all removed the pattern of the injected lymphatic vessels, which formed a plexus on the dorsal side of the vein, was plainly visible. From this plexus a vessel extended posteriorly for some distance parallel with the direction of the postcava and gradually passed through the wall of the postcava at a very small angle, to form a tap on the dorsal side, a little in front of the anterior level of the lumbar nodes. The point of the tap was easily seen as the hard injection mass projected out into the vein and was continuous with that in the lymphatic vessel. Contrary to expectations, the direction of the vessel approaching the tap and the direction of the mass projecting from the tap was opposite to that of the flow of the blood in the vein.

In the dissection of the renal vein the results were not so good, as none of the mass had remained in the lymph vessel approaching the tap and the point of entrance could not be determined.

At two other points in a number of specimens the injection mass was observed to appear in the veins. One was in the portal vein and the other was in the right iliolumbar vein near the body wall. While it is probable that venolymphatic taps occur at these points, still no such absolutely definite evidence of their presence was obtained as in the case of the postcaval and renal taps. For instance, in the case of the portal vein, very frequently the mass passed into it when the injection was made from the superior mesentric nodes, yet while almost complete injections were often obtained in the superior mesenteric nodes by the mass backing up into them from the cisternal region during injection from the anterior inguinal nodes, still never in the case of this sort of injection did the mass pass into the portal vein, or if it did, it was in such small amounts as not to be noticed. It is felt that more work is necessary on these points before a definite statement can be made in regard to their presence.

LYMPH VALVES

Lymph valves are very numerous in all main lymph vessels, as from the cervical nodes to their venous taps, or in the thoracic duct or from the lumbar nodes to the cisterna chyli, but they do not seem to be present at all from the cisterna chyli into the intestinal nodes. Due to this fact, practically complete injections of the intestinal nodes have been obtained from injections made in the inguinal nodes which resulted from the mass backing up into the intestinal nodes from the cisterna chyli.

The presence of a valve is evidenced by a constriction in the lymph vessel, so that if there are many valves present the vessel, when filled, has the appearance of a series of bulges.

CONCLUSION

Two points at least ought to be emphasized which are unusual in this animal. They are the jugular lymph sac and the renal and postcaval venolymphatic taps.

The presence of the jugular lymph sac is an unusual structure in adult anatomy and perhaps is but a remnant of the embryonic condition. The embryology of this form has not as yet been worked out, however, so this structure is interesting merely from the speculative standpoint.

The renal and postcaval taps may be more common than their demonstration frequency would indicate, though that is largely conjecture. However, the appearance of the postcaval tap was a total surprise, while that of the renal tap was rather expected, since in a number of specimens the injection mass passed out through the lymphatics toward the renal vein as described, though in only one case did it actually appear in the renal vein.

The fact of the presence of these taps even in so small a percentage of the animals as this study would indicate is additional proof that the venolymphatic taps of the jugulosubclavian region are not always the only points through which lymph is returned into the blood.

Occasion is taken here to express my appreciation for the kindly interest and assistance rendered by Doctor Stromsten, under whom these studies were made.

A SUMMARY OF A MONOGRAPH ON THE MORPHOLOGY OF THE LYMPHATIC SYSTEM IN THE ANURAN AMPHIBIA, WITH ESPECIAL REFERENCE TO ITS ORIGIN AND DEVELOPMENT¹

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The writer has devoted much time during the past five years to a study of the lymphatic system in Amphibia and has brought together the results in a monograph, illustrated by many figures and plates. The first half was ready for publication in August, 1917, but on account of the war and its attendant difficulties and unavoidable delays, it was not to appear until the coming March (1919). Meanwhile, the second half has been completed, and since it would be more logical and satisfactory to publish the two parts together, the first was withdrawn and has been combined with the second part in a single treatise. Present circumstances will not permit its early appearance in print, and because a great many of the data were gained several years ago, he deems it expedient to furnish a summary of it now for the benefit of other investigators.

Larval and adult specimens of *Bufo vulgaris*, *B. lentiginosus* and *Rana pipiens* constituted the material used in the investigation. The facts which the author's researches revealed and the conclusions which have been reached are briefly outlined as follows:

1. ON THE LYMPHATIC SYSTEM IN FULLY FORMED INDIVIDUALS

1. In the neighborhood of the lymph hearts, valves guard the openings between adjacent lymph sinuses.

2. The anterior lymph heart, paired, is situated dorsal to the transverse process of the third vertebra, and it communicates

¹ Ready for publication, January 1, 1919.

by means of a valve with the anterior vertebral vein, a short tributary of the internal jugular vein. In a young specimen of *Bufo lentiginosus*, soon after its metamorphosis, there were five valvular apertures between the lymph heart and the bordering lymph sac (*sinus subscapularis*).

3. The single pair of posterior lymph hearts in a young individual of *Bufo lentiginosus* is slightly smaller than the anterior (anterior heart, 0.45 mm.; posterior, 0.42 mm.); each joins the posterior vertebral vein of the corresponding side, a branch of the ischiadic vein, and each possesses four afferent pores.

4. In an adult *Rana pipiens* two posterior lymph hearts were present on the right side and three on the left, the third or most caudal one being very small and partly fused with the next one in front; each one of these hearts, the vestigial one included, has its own ostium venosum. The larger and functional hearts have from twenty to thirty afferent portals.

5. The wall of a lymph heart is composed of three coats: 1), a tunica interna, formed of typical endothelial cells; 2), a tunica media, which contains muscle bundles interlaced in a complex manner; 3), a tunica externa or adventitia, which binds the lymph heart to the surrounding tissues.

6. Sometimes a trabeculum is found stretching across the cavity of the lymph heart.

2. ON THE MODIFICATION OF THE VENOUS SYSTEM DURING DEVELOPMENT

1. The primary venous ground-plan of anuran embryos is similar to that of other vertebrate embryos.

2. The rôle played by the subcardinal veins in the formation of the postcava corresponds closely to that in other vertebrates.

3. By the degeneration of the anterior segment of the postcardinal vein, the pronephric sinus (the plexus of venous sinuoids encompassing the pronephros and originally constituting the junction of the pre- and postcardinals and Cuvierian duct) dwindles in size and becomes the proximal segment of the precardinal or internal jugular vein.

4. The primitively symmetrical system of intersegmental veins undergoes radical modifications, at first producing the vein of the lateral line by longitudinal anastomoses, and subsequently differentiating into the anterior and posterior vertebral and lumbar-dorsal veins.

5. The anterior and posterior lymph hearts of the toad (*Bufo vulgaris* and *B. lentiginosus*) develop in relation to the third and eleventh intersegmental veins, which drain into the postcardinal. These tributaries ultimately become parts of the anterior and posterior vertebral veins, which, in consequence of the reduction of certain segments of the embryonic venous plan and the marked shifting of relations that occurs during development, come to be branches of the internal jugular and ischiadic veins, respectively.

3. ON THE COMPONENTS OF THE LYMPHATIC SYSTEM IN 15-MM. TADPOLES (*BUFO*)

1. A large sinus, the primary maxillary lymph sinus (*sinus lymphaticus primarius maxillaris*), which may be divided into circumoral, mandibular, temporal, and paracardial divisions, is situated in the ventral and lateral regions of the head.

2. A jugular lymphatic (*lymphatica jugularis*),² one on either side, runs parallel to the proximal portion of the internal jugular vein and connects the primary maxillary sinus and the anterior lymph heart.

² At the present time a few investigators, the writer included, are using the adjective 'lymphatic' (Latin, *lymphaticus-a-um*) also as a noun to designate the larger lymph channels. The lymphatic system, constituting the third major subdivision of the vascular system, the term 'lymphatic,' used instead of 'lymph duct,' seems more specific and to accord better with the terms 'vein' and 'artery.' In his present work the writer has also carried this idea over into the scientific terminology. The expressions '*vas lymphaticum*' and '*truncus lymphaticus*' have been generally used to designate the important lymph vessels. The writer, on the other hand, has dropped the word '*vas*,' signifying vessel, and has taken over the Latin adjective in its feminine form, '*Lymphatica*,' as a substantive substituting it as the generic name for the larger lymph channels or ducts, because it conforms with the terms '*vena*' and '*arteria*.'

The Latin word '*lymphatus*,' meaning practically the same as '*lymphaticus*,' might be employed as '*lymphata*,' which being shorter than '*lymphatica*' would be preferable, but the latter term, being more familiar, is more acceptable.

3. The pair of anterior lymph hearts (*corda lymphatica anteriora*) lie lateral to the third and fourth myotomes at the level of the third spinal ganglia and join the rudiments of the anterior vertebral veins at the junction of the latter with the pronephric venous sinuses. On the opposite or dorsal side of each heart a single afferent gateway is present.

4. The lateral lymphatic of the trunk (*lymphatica lateralis corporis*) courses along the myotomes between the anterior and posterior lymph hearts.

5. The paired subvertebral lymphatic (*lymphatica subvertebralis*) passes from the anterior lymph hearts medially, thence along the aorta to the caudal end of the trunk, where they bend lateralward to unite with the lateral lymphatic in the vicinity of the posterior lymph heart.

6. A dorsal lymphatic (*lymphatica dorsalis corporis et caudalis*) exists in the midline above the neural tube and stretches from the anterior region of the trunk to the tip of the tail; it connects with the lateral lymphatics by transverse branches.

7. The posterior pair of lymph heart anlagen lie at the level of the eleventh spinal ganglia against the posterior vertebral veins, but in 15-mm. embryos have not yet established a communication with these veins.

8. The lateral lymphatic of the tail (*lymphatica lateralis caudalis*) is a direct continuation of the lateral-line lymphatic of the trunk.

9. The ventral caudal lymphatic (*lymphatica ventralis caudalis*) begins as a paired vessel at the point of confluence of the subvertebral and lateral lymphatics and is prolonged into the tail, converging and fusing with its fellow further back.

10. The lymphatic extensions of the fore and hind extremities (*lymphaticae brachialis, iliacis et femoralis*) appear later.

11. Lymph capillary plexuses springing from tributaries of the large deep lymph ducts, named above, traverse the subcutaneous tissue.

4. ON THE ORIGIN AND DEVELOPMENT OF THE PRIMARY MAXILLARY LYMPH SINUS

1. The anlagen of the primary maxillary lymph sinus first become visible in 5-mm. embryos (*Bufo vulgaris*) as small, solid and discontinuous cell masses frequently attached to the wall of a bilateral haemal channel, the potential inferior jugular vein which is at this time a component of the primitive vascular plexus ventral to the oral and pharyngeal cavities.

2. The writer is somewhat in doubt regarding the significance of the adherence of some of the maxillary sinus anlagen to the potential inferior jugular vein during their early genetic stage. Are they derivatives of the haemal endothelium, or are they analogous to the extra-intimal lymphatic spaces of mammalian embryos? (Aside from the fact that the view of the discontinuous mesenchymal origin of all vascular anlagen, irrespective of whether haemal or lymphatic, has been shown to be fairly conclusive in recent investigations, the blood vascular plexus, just referred to is still indifferent, that is, has not clearly differentiated into arteries and veins, at the time when the lymphatic anlagen adhere to it. It is conceivable how at such an early developmental stage, the actively proliferating endothelium of the primitive vascular plexus may also assist in the production of the third set of vascular channels, the lymphatics.)

3. The discrete lymphatic anlagen of the primary maxillary lymph sinus lose their contact with the veins, acquire lumina, elongate and enlarge, and proliferate extensions, some of which coalesce with one another of the same side, and others project ventromedially to connect with those from the other side, thereby creating an intricate network of lymph vessels just below the external jugulars and carotids.

4. This lymphatic network, representing the pars mandibularis or principal portion of the latent sinus, gives rise to the other divisions, the circumoral, the temporal, and the paracardial, by outgrowths forward, lateralward, and backward.

5. During these developmental stages the endothelium of the vascular channels and anlagen, both haemal and lymphatic, can be distinguished plainly from the surrounding mesenchymal cells by its possession of a greater number of yolk globules.

6. The lymphatic plexus gradually becomes converted into a spacious and uninterrupted lymph reservoir by the expansion and fusion of its numerous components. In this genetic process, the interstices of the circumjacent mesenchyme do not participate, for the outlines of the sinus remain sharply defined during its multilocular condition.

7. During the period of its active formation the primary maxillary lymph sinus is nowhere in open connection with other vascular channels, neither with blood nor with lymph vessels. Its appearance of great distention is doubtlessly due to the lymph, which, permeating the tissues, accumulates within it in rapidly increasing volume, there being no outlet. Blood cells are not present in its cavity at any time.

8. A communication is established between the temporal divisions of the primary maxillary sinus and the jugular lymphatics, and thus indirectly with the anterior lymph hearts, in approximately 8- to 9-mm. embryos (*Bufo vulgaris*).

5. ON THE ORIGIN AND DEVELOPMENT OF THE JUGULAR LYMPHATICS AND THE ANTERIOR LYMPH HEARTS

1. The jugular lymphatic and the anterior lymph heart have their origin in a venolymphatic plexus, derived from the first three intersegmental veins which are an integral part of the early vascular system of the embryo.

2. All connections between the veins (pronephric venous sinus) and the plexus are lost, except those of a demarcated and more close-meshed portion of the plexus which represents the anlage of the lymph heart.

3. The first definite indications of the heart anlage can be observed in 4- to 5-mm. embryos (*Bufo vulgaris*).

4. The simple globular chamber of the anterior lymph heart is produced by the dilation and confluence of the channels of its antecedent plexiform phase and by the further distention of the single cavity so formed.

5. The remainder of the venolymphatic plexus becomes a lymphatic one by its detachment from the vein; in it a conduit becomes dominant which extends forward to the pars temporalis of the primary maxillary lymph sinus as the jugular lymphatic.

6. The developing lymph heart temporarily breaks away from the surrounding lymphatic plexus.

7. A union is secondarily reestablished between the lymph heart and the common segment of the jugular and lateral-line lymphatics. This is effected by the coincident expansion of the caliber of both structures which brings about an intimate contact between them and so makes possible the development of a portal from a rupture in the intervening partition. At this point the afferent valve is formed by a thickening of the endothelial cells; later, more such portals arise in the manner indicated.

8. The efferent valve is developed from oppositely placed endothelial cushions in the lumen of the lymphaticovenous junction.

9. Blood cells are found in the heart until the valves have been laid down and its pulsations have commenced.

10. The characteristics of certain cells during the transformation of the plexus into the single-chambered lymph heart suggest a concomitant haemopoietic process.

11. The muscle coat of the lymph heart is derived from the bordering mesenchymal cells, which become fusiform and modified into contractile fibers and gradually acquire a parallel arrangement in a distinct layer.

6. ON THE ORIGIN AND DEVELOPMENT OF THE LATERAL LYMPHATICS OF THE TRUNK

1. A double series of discontinuous lymphatic anlagen appears in the lateral axial region of the trunk throughout its entire length, one row situated along the longitudinal anastomosis (lateral-line vein) of the intersegmental veins, and the other along the postcardinal vein.

2. When the lymphatic anlagen can first be clearly recognized as such, they lie as small definitely walled spaces, either against the intima of the venous channel or in close proximity to it.

3. By their elongation and fusion two lymph channels result, the lymphatic of the lateral line (*lymphatica lateralis lineae corporis*) and the juxtacardinal one (*lymphatica juxtacardinalis*).

4. The two lymph conduits, at first independent of one another, become joined as they sprout numerous branches and produce a luxuriant plexus lateral to the myotomes.

5. This lymphatic plexus establishes continuity with the lymphatics in the territory of the anterior lymph heart; in fact, the extreme anterior portion of the plexus is derived from the venolymphatic one from which the jugular lymphatic and the lymph heart are developed.

6. In the lateral lymphatic plexus, a vessel becomes paramount by distention and the incorporation of collateral vessels, and it consequently transmits more and more of the lymphatic stream; this vessel has been termed the lateral lymphatic of the trunk (*lymphatica lateralis corporis*).

7. ON THE ORIGIN AND DEVELOPMENT OF THE SUBVERTEBRAL LYMPHATICS (THORACIC DUCTS)

1. The rudiments of the paired subvertebral lymphatic, when first distinguishable as such, exist as consecutive spindle-shaped spaces, located lateral to the aorta and joined to slender medial extensions of the juxtacardinal lymphatics.

2. Acquiring continuity by the end-to-end union of its rudiments, the lymph duct is at first very much attenuated, but progressively becomes more conspicuous in section by the marked expansion of its lumen.

3. Haemopoiesis occurs in the axial mesenchyme; this is especially true in the iliac region where blood cells in different stages of development are scattered throughout the mesenchyme and make their way into lymph vessels, thus demonstrating the accessory haemophoric function of the embryonic lymphatics.

8. ON THE ORIGIN AND DEVELOPMENT OF THE POSTERIOR LYMPH HEARTS

1. The first sign of the origin of the posterior lymph heart manifests itself in 10- to 11-mm. embryos (*Bufo vulgaris*) at the level of the eleventh spinal ganglia as an accumulation of mesenchymal cells in a small circumscribed area encompassing certain channels of the lateral lymphatic plexus which lie against the potential posterior vertebral vein.

2. The lymph channels within the zone of the mesenchymal condensation become even more plexiform, after which by their

widening and complete fusion along their apposed surfaces they produce a single unbroken cavity, the chamber of the lymph heart.

3. During the foregoing genetic changes the anlage of the lymph heart is nowhere in direct and open communication with the contiguous vein.

4. The original connections (approximately seven in number) between the lateral lymphatics and the lymph-heart anlage become constricted and break away. The isolation of the heart cavity is only a transitory one, for one or two junctions are soon reestablished where lymph heart and the nearest lymph vessels come into juxtaposition as a result of their expansion.

5. The valves of the afferent portals of the heart chamber originate in the same way as do those of the anterior lymph heart. The ostium venosum, or lymphatico-venous tap, on the contrary, is formed by the thinning out of the wall between lymph heart and vein, followed by the growth into the venous lumen of an endothelial projection, which becomes hollow, thereby producing the aperture as well as the margins of the teat-like valve.

6. During its development, the posterior lymph heart is an haemopoietic focus; blood cells are formed within it, and still others pass through it on their way to the blood stream.

7. The muscular tissue of the heart wall is derived from the original aggregation of mesenchymal cells.

8. Comparing the formation of the anterior lymph heart with that of the posterior lymph heart, a considerable difference is evident. Whereas the former arises early in embryonic development and is differentiated from a venolymphatic plexus, which is part and parcel of the primitive circulatory plan, the latter appears relatively late and is differentiated from a lymphatic plexus at that time clearly sequestered from the haemal organization.

9. In an advanced larva of *Rana pipiens* there were three posterior lymph hearts on both sides, corresponding to the eleventh, twelfth, and thirteenth intersegments; another larva, only slightly older, possessed two on each side, thus demonstrating that a difference in the number of such hearts may exist in the same species.

10. Variations may also occur in the same individual, as evidenced by the finding of a normally sized posterior lymph heart on one side and a small one on the opposite side in a tadpole of *Bufo lentiginosus* (also compare section 1, 4).

9. ON THE ORIGIN AND DEVELOPMENT OF THE DORSAL,
LATERAL, AND VENTRAL LYMPHATICS OF THE TAIL

1. The dorsal lymphatic has a bilateral origin in the trunk, beginning as a double series of spindle-shaped anlagen located dorsal to the neural tube.

2. When the successive anlagen have become continuous, the two channels combine into a single one; its posterior end then grows caudally into the tip of the tail by the proliferation of its endothelium.

3. The lateral caudal lymphatic represents a caudal prolongation of the similarly situated duct in the trunk.

4. The ventral caudal lymphatic develops as a growth backward from the confluence of the subvertebral and lateral ducts of the trunk; at first paired, it fuses into a single structure except at its proximal end.

10. ON THE FORMATION OF THE LYMPHATIC CAPILLARIES

1. After the larger lymph ducts are laid down by the coalescence of originally separate anlagen, they elongate by the proliferation of their intimal cells and bud branches, which grow centrifugally and form the lymph capillary plexuses.

2. The observation of the sprouting and centrifugal growth of the lymphatic capillaries cannot be used as an argument against the theory of the in situ and discontinuous origin of the larger systemic lymph conduits.

11. ON THE TRANSFORMATION OF THE LYMPHATIC VESSELS OF
THE TADPOLE INTO THE LYMPH SACS AND
SINUSES OF THE ADULT

1. Three variations in method can be recognized in the formation of the definitive lymph sacs: 1), the conversion of an antecedent lymph capillary plexus with the possible participation of

the circumjacent mesenchyme; 2), the simple adaptation of an embryonic sinus to late larval and adult conditions; 3), the marked distention of a duct.

2. All of the deep lymph sinuses of the mature animal are derivatives of the large lymphatic channels of the embryo; all of the superficial sacs, with the exception of the submaxillary and temporal, have their origin in the most terminal or subcutaneous branches of these channels.

3. The submaxillary and temporal lymph sacs are directly derived from the mandibular and temporal divisions of the primary maxillary lymph sinus, the sublingual, hyoidal, pulmonary and sternal sinuses originate as diverticuli of the paracardial divisions, and the large unpaired basilar sinus develops from a bilateral extension forward of the junctions of the paracardial and temporal divisions of the primary maxillary sinus and the jugular lymphatics.

4. The paired subscapular sinus of the adult is in part derived from the posterior portions of the paracardial and temporal divisions of the primary maxillary sinus and in part from the entire jugular lymphatic, which explains its intimate relation to the anterior lymph heart. During development, it becomes divided from the temporal lymph sac by the formation of the scapula and its musculature.

5. The two subvertebral lymphatics, having come in apposition by their dilation, combine into a single channel, which by further expansion becomes an extensive sinus. In this genetic process, it absorbs the lateral lymphatics and their deep tributaries.

6. The dorsal lymphatic of the trunk undergoes regression during the later larval period, but a small vessel probably persists to drain the neural canal and its contents.

7. The mesenteric lymphatics and the deep sinuses of the posterior portion of the abdominal cavity, such as the pelvic, pubic, periproctal, etc., arise as extensions and evaginations of the subvertebral sinus and insert themselves between the viscera.

8. The iliac sinus, adjacent to the posterior lymph heart in the mature individual, corresponds to the influence of the subvertebral, lateral, and ventral caudal lymphatics in the larva.

9. All of the superficial lymph sacs (except the submaxillary and temporal) of the head and trunk, such as the supra-orbital, craniodorsal, lateral, abdominal, and pectoral sacs, as well as those of the extremities, have their beginning in the subcutaneous lymph capillary plexus, derived from tributaries of the dorsal and lateral lymph ducts of the trunk.

12. ON THE HOMOLGY OF THE CHIEF COMPONENTS OF THE
LYMPHATIC GROUND-PLAN IN THE DIFFERENT
GROUPS OF VERTEBRATES

1. Among vertebrate animals three regions of lymphaticovenous communications can be recognized: an anterior, a middle, and a posterior region. The anterior or jugular one is most constant, being present in all classes and orders of vertebrates.³ In the tailed amphibians (Urodeles and Gymnophionia) the numerous metamerically arranged lymphaticovenous connections of the lateral line, extending from the jugular to the caudal regions, do not permit such a definite division into regions. The middle group of taps have been clearly demonstrated in Mammals (Primates, Marsupials, Rodents). The posterior or caudal lymphaticovenous junctions are present in Fishes, Amphibia, Reptiles, Birds, and perhaps in the embryos of Mammals.

2. The multiple posterior lymph hearts of certain Anura must be considered as a persistence of a number of the segmental lymph hearts found in the more primitive Amphibia, the Urodeles and Gymnophionia.

3. The writer regards the caudal hearts, both venous and lymphatic, of Fishes, the posterior lymph hearts of Amphibia, Reptiles, and Birds, and the vestigial posterior or iliac sacs of mammalian embryos as homologous structures.

4. The author believes he has morphological evidence to show that the posterior portions of the jugular lymph sacs of Mammals, Birds, and Reptiles are identical with the anterior lymph hearts of Anura.

³ References to the literature will be cited in the monograph.

5. Topographical relations and genetic data show that the primary maxillary lymph sinus of anuran tadpoles corresponds to the subocular lymph sinus of Fishes.

6. The paired subvertebral lymphatic is represented in all classes of vertebrates, but different names are applied to it, such as, thoracic ducts, periaortic sinus, prevertebral lymphatics, abdominal sinus, etc.

7. The bilateral jugular lymphatic (cephalic duct, truncus jugularis, lateral pharyngeal lymphatic) is present in all groups of vertebrates.

8. The lymphatic of the lateral line evidently is of constant occurrence in all Fishes, Gymnophionia, and Urodeles, in the tadpoles of Anura, in the embryos of Reptiles and Birds, and the writer believes vestiges will eventually be found in mammalian embryos.

9. The dorsal lymphatic is a common feature of Anamia; the embryos of Amniotes have not been sufficiently studied in this respect.

10. A number of the superficial lymph channels in Fishes and in the Amphibia can be readily homologized, but this is impossible in the higher vertebrates.

Resumen por el autor, George Washington Tannreuther.
Universidad de Missouri.

Duplicación parcial y completa en los embriones de gallina.

Los embriones que se describen en el presente trabajo han sido seleccionados en una colección hecha durante un periodo de diez años. A. El estado más joven observado, en el cual existen dos o más embriones, está representado por un blastodermo con cuatro líneas primordiales, dos de las cuales se extienden anteriormente y las otras dos posterior y lateralmente encontrándose sus extremos posteriores en una región común. Las líneas primitivas no presentan desviación alguna de la estructura normal. Un estado ulterior de duplicación completa está representado en un blastodermo con dos embriones normales completamente independientes, correspondiente al comienzo del segundo día de incubación. Los dos embriones difieren algo en su estado de desarrollo. B. Duplicación parcial: 1). Un blastodermo próximamente a las 21 horas de incubación presenta un embrión con una sola línea primitiva, la cual termina anteriormente en dos nudos de Hensen, dos procesos cefálicos y la formación temprana de los pliegues neural y cefálico. 2). En otro caso un embrión está representado por una sola cabeza y región del cuello, que termina posteriormente en dos troncos distintos. 3). Un caso de anormalidad extrema está representado en un blastodermo en el cual dos embriones poseen regiones del tronco normales las cuales son continuas en sus extremos anteriores y presentan las regiones cefálica y del cuello comprimidas anormalmente. 4). Un embrión próximamente a las 68 horas de incubación presenta dos corazones normales claramente separados. El autor incluye en el texto diversas figuras que demuestran las anormalidades descritas.

PARTIAL AND COMPLETE DUPLICITY IN CHICK EMBRYOS

GEORGE W. TANNREUTHER

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SIX FIGURES

It is not an unusual thing to find abnormalities of varying degrees in chick embryos. Especially is this true in the case of poorly regulated incubators. Development may begin in a perfect normal way, but a low or a high temperature often causes abnormal growth in later stages. The question, how a single blastoderm possesses the potency to produce one or several embryos, is as yet an unsolved problem. It has been proved experimentally, in some animals at least, that two or more embryos can be produced from the parts of one ovum. In no instance, however, has it been demonstrated that the number of individuals resulting from a single blastoderm can be controlled. Until some plausible explanation is given for the splitting up of the blastoderm into several equipotent regions, we can merely give a description of these unusual departures as they are found from time to time in the embryology of the different vertebrates.

In the armadillo, normally, four or more embryos result from a single fertilized egg. The separation of the embryonic rudiments becomes visible after the formation of the two primary germ layers. In the chick, normally, but a single embryonic rudiment appears on the blastoderm. In the case of the chick, partial duplication of parts is frequent, but the formation of two or more complete embryos on a single blastoderm is a rare occurrence.

The following paper is a brief description of a few very unusual chick embryos, which may be considered of considerable value or importance, especially for the embryologist, who is more directly interested in the occurrence of polyembryony as it may be found in the different groups of vertebrates.

The embryos were fixed in picrosulphuric killing reagent, stained in alum-cochineal, cleared in xylol, and mounted in damar. The embryos were sufficiently transparent that the various structures at different levels could be followed with a considerable degree of accuracy. The embryos were collected and preserved during a period of ten years. Those with minor or slight abnormalities were discarded, while those of unusual formations were mounted and preserved.

Figure 1 represents the earliest stage in which abnormalities were observed. The area opaca and area pellucida do not vary much from that of the normal. The four primitive streaks fuse at a common point (C.F.Pr.St.), which from all appearances represents the posterior ends of the four structures. The primitive streaks 1 and 2 are perfectly normal and correspond to an embryo of about seventeen hours. In either case the primitive folds and groove are well marked and show a distinct primitive pit at their anterior ends. Hensen's node is rather faint. The head process and the head fold in either case are distinctly normal and show no departure from the usual condition. The formation of the three germ layers compares to that of the typical embryo at the same stage of development. The primitive streaks 3 and 4 are much larger than 1 and 2 and more widely separated. The primitive folds, grooves, and pits are better developed than the two smaller streaks. The head process in either case is very faint and almost indistinguishable. The head folds are well developed. The primitive streaks 3 and 4 near their common point of fusion are poorly developed and do not show the presence of folds at all. The mesoderm in the region equidistant from either streak is well developed and gives the appearance of a distinctly drawn-out shaded area (*Mes.*). The germ layers have kept equal pace with the streaks in their development. The primitive streaks 3 and 4 might be taken as the posterior extensions of 1 and 2, but when we take into consideration their well-developed structures corresponding to the conditions found in the normal primitive streak, we can undoubtedly regard them as distinct primitive streaks.

Figure 1 in all probability represents a blastoderm which has

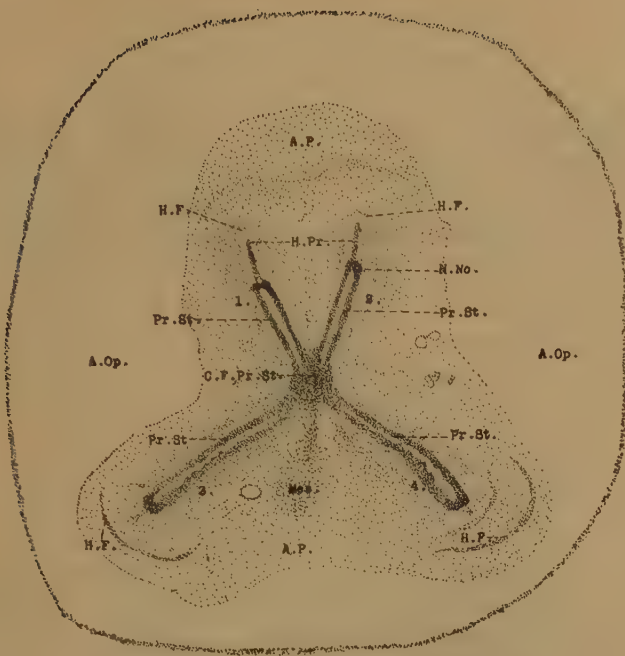


Fig. 1

All figures were made with the aid of a camera lucida, at table level with a 16-mm. objective and a no. 6.4 eye-piece. The front lens of the objective was removed. All drawings are about the same magnification and reduced four-sevenths.

ABBREVIATIONS

Ao.A., aortic arch
Ab.Br., abnormal brain
A.C.S., anterior cerebral suture
A.C.V., amnio cardiac vesicle
A.I.P., anterior intestinal portal
Al., allantois
Am., amnion
A.Op., area opaca
A.P., area pellucida
A.Vas., area vasculosa
Au.Ves., auditory vesicle
B.Is., blood islands
C.F.Pr.St., common fusion of primitive streaks
C.Pr.St., common primitive streak
Ec., ectoderm
Ent., entoderm
F.B., fore brain
F.G., fore gut
F.L., fore limb
G.C., gill cleft
H.B., hind brain

H.F., head fold
H.G., hind gut
H.N., Hensen's node
H.Pr., head process
Ht., heart
I.Gr., intestinal groove
Mes., mesoderm
Mes.So., mesoblastic somite
M.B., mid brain
Nch., notochord
N.F., neural fold
Op.Ves., optic vesicle
Pr.Am., pro amnion
Pr.Gr., primitive groove
Pr.Pl., primitive plate
Pr.St., primitive streak
S.T., sinus terminalis
T.F., tail fold
Tr.Ars., truncus arteriosus
V.Ao., ventral aorta
Vt.Ar., vitelline artery
Vt.V., vitelline vein

become divided into four equipotent regions. The question arises, if the blastoderm with the four primitive streaks continued to develop, would the four resulting embryos be independent or united into a single monster. Judging from the conditions found in figure 3, four distinctly independent embryos would have resulted.

Figure 2 represents an embryo of about twenty-one hours' incubation. The area opaca, pellucida, and vasculosa are more

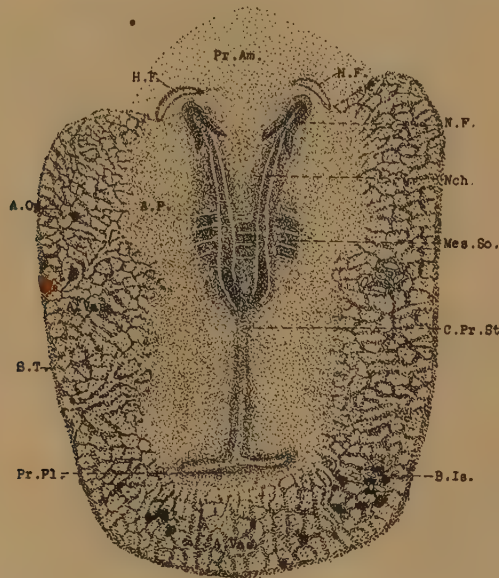


Fig. 2

elongated than in the normal blastoderm. The early formation of the vascular area with the sinus terminalis does not show any departure from the normal course of development. The blastoderm shows a single primitive streak, terminating at its anterior end with two Hensen's nodes and head processes. The embryo anterior to the primitive streak is represented by an almost complete duplication of parts. The development of the head folds, the neural tubes, and the notochords correspond to the typical conditions. The head regions are distinctly inde-

pendent, but a fusion occurs in the somite region, where the middle paraxial mesoblast with its somites is common to both of the notochords. In figure 2, if development had continued until the end of the incubation period, no doubt an embryo with two distinct heads and neck regions with a common trunk would have resulted.



Fig. 3

The blastoderm in figure 3 shows a well-developed vascular area, with a distinct sinus terminalis. The anterior vitelline vein, corresponding to either embryo, has begun its early development. With the exception of a very small part of the posterior end of the primitive streak, there is a complete duplication of structures on a common blastoderm. There is no means of

determining from a study of the two embryos why one has reached a further stage in its development than the other. The formations in either embryo do not show any departure from the normal course of growth. The head folds in either case are distinct and show the fore gut well developed. The amnion and tail folds of either embryo have begun their early stages of development.

In the early period of incubation the two embryos began as two primitive streaks with their posterior ends fused. The persistence of the fusion is well marked, as indicated in figure 3 (*C.Pr.St.*). Judging from the independent formation of the two tail folds, as shown in the figure, two distinctly independent chick embryos would have resulted at the end of the incubation period. The size of the yolk and egg from which the blastoderm was taken corresponded to that of the average in the lot.

If the progress of development in figure 3 can be taken as a safe criterion, we could readily foretell the resultant development in figure 1, where the posterior ends of the four primitive streaks show a common connection. As in figure 3, an independent embryo would have resulted from each of the primitive streaks. Thus producing four individuals on a single blastoderm.

The embryo represented in figure 4, no doubt began its development as two independent primitive streaks, with a later connection or fusion of the anterior ends of the two head processes. The embryo shows a complete duplication of the trunk region and the vitelline veins. The development of the head region is normal. It shows the differentiation of the anterior end of the neural tube into the primary brain vesicles and the early formation of the optic vesicles. The head fold is well developed and represents the demarcation of the head region from the blastoderm. The amnion shows the usual course in its early development. The heart shows the characteristic development of a thirty-two-hour embryo. The ventral aortae extend to the anterior end of the fore gut, where they continue as the aortic arches to the dorsal side of the gut and extend posteriorly, becoming the two dorsal aortae. The ventral aortae, first pair of aortic arches, and the dorsal aortae are unusually well developed

and easily followed. There was no indication of the early development of a second pair of aortic arches. The two dorsal aortae are widely separated on the dorsal side of the fore gut, and on reaching the double trunk region, either aorta continues posteriorly on its corresponding side. Thus either trunk region has but a single dorsal aorta. There were no indications of either dorsal aorta dividing in the trunk region. The vitelline veins



Fig. 4

are in their early stages of development. The anterior vitelline veins are present. There are four lateral vitelline veins. The anterior pair, right and left, are of the usual type as found in the normal embryo. They are well developed, as indicated in the figure. The accessory pair of vitelline veins extend anteriorly on the blastoderm between the two trunk regions and enter the sinus venosus on the median ventroposterior end. Thus, either

trunk region has two vitelline veins, the normal number. The circulatory system taken in its entirety, with the exception of the vitelline veins and arteries, does not show any unusual developments.

The fore gut, as shown in the figure, is a single closed tube anterior to the somite region, but at its posterior end the gut becomes divided into a right and a left posterior extension. This division is due to the presence of the median splanchnic folds. Two anterior intestinal portals instead of one is formed, as indicated in figure 4 (*A.I.P.*). Thus there is formed a gut cavity corresponding to either trunk region. A slightly later stage undoubtedly would show the median splanchnic folds much better. The notochord is duplicated in the trunk region, but continues anteriorly as a single structure. The primitive streaks and Hensen's nodes are well developed.

The anterior end of the neural tube corresponds to the condition found in the normal embryo, but at the point where the division into a right and a left extension occurs, it is abnormally wide in a transverse plane. The accessory neural folds of the trunk region, which form the inner half of either neural tube, are continuous at the point where they form the posterior limit of the undivided part of the tube. The early stages of development in the tail-fold regions are well marked. The dotted outline shows the limit of the area pellucida. The embryo began as two independent primitive streaks, but complete development would have resulted in an individual with a single head, a single neck, and a double trunk.

Figure 5 represents a very unusual abnormality. It would be rather difficult to conjecture the conditions as they occurred in the first stages of development. In all probability, the embryos began as two independent primitive streaks, with the anterior ends of the head processes continuous or in immediate contact. With the exception of the shape of the pellucida area, the blastoderm shows no departure from that found in the normal individual. The sinus terminalis was well developed and showed its termination anteriorly into the right and left anterior vitelline veins, which were continuous with heart number (2).

The anterior ends of the neural tubes are condensed into a very small region. The brain (*Ab.Br.*) of either embryo is composed of a series of indefinite folds and rudimentary vesicles. There are no structures present that could be considered as optic vesicles or as any definitely marked formations. The neural tube in either trunk region is well developed and corresponds to the normal embryo of about forty-two hour's incubation. There is a distinct notochord in either trunk region.



Fig. 5

A common fore gut is present beneath the abnormal brain and is limited laterally by the splanchnic folds on either side of the head region (*Ent.Fo.*). The fore gut opens in either direction and the anterior intestinal portal of either embryo is situated near the anterior end of either somite region. Thus the anterior end of either fore gut is a common continuous structure. The tail folds and hind gut in either case have just begun their development and agree with the conditions in the normal developing chick.

The area vasculosa is unusually well developed. The upper side of the figure corresponds to the anterior end of the extra-embryonic blood system. The hearts (1) and (2) are normal in every respect, and possess a distinct well-developed truncus arteriosus (*Tr.Ars.*). Either truncus forks right and left and give rise to the ventral aortae (*V.Ao.*), which continue dorsally around the fore gut in either embryo and give rise to the dorsal aortae. The two dorsal aortae of either embryo are independent, except at their anterior ends. The two hearts, when the blastoderm was removed from the egg, did not pulsate simultaneously, but in a regular alternate order. The blood passed directly from the ventral aortae on either side through the aortic arches to the corresponding dorsal aortae, which continue independently in the trunk region. The two vitelline arteries in either embryo are distinct and show no unusual variations. Two well-developed vitelline veins are present on either side, which unite anteriorly to form the sinus venosus of the corresponding heart. Thus the vitelline veins of either heart pass out in the splanchnic folds of different anterior intestinal portals.

Either heart, as represented in figure 5, no doubt begun as a distinctly independent formation, two endocardial tubes being present in the early development of either structure. This belief is further substantiated by the fact that two distinct vitelline veins are present in either case.

A distinct common amnion (*Am.*) has begun its development, which partially covers heart (2). In all probability the amnion would have continued as a common structure for both abnormal embryos. One of the trunk regions is slightly larger than the other with a few extra somites. Figure 5 shows a complete duplication of the heart, vitelline veins, vitelline arteries, and trunk regions. End development of the embryos in figure 5 would have produced a very unusual chick monster.

Figure 6 represents an embryo of sixty-eight hours' incubation. The blastoderm with its well-developed vascular area does not show any departure from that of the normal developing individual. The sinus terminalis with its two anterior vitelline veins is not represented. The brain shows considerable ab-

normal development. The primary brain vesicles are present, but they are very much distorted. The optic vesicles are unusually small and poorly developed. The crystalline lenses, if present, cannot be recognized in the whole mount. The audi-

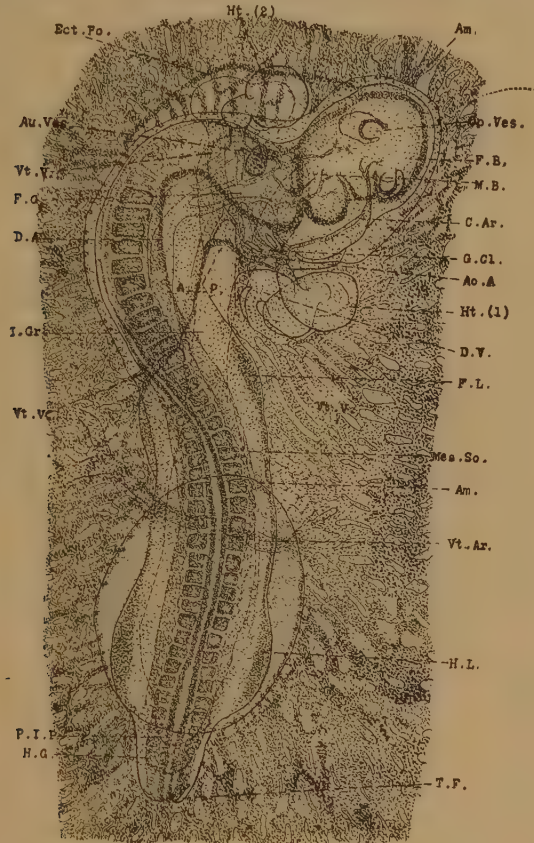


Fig. 6

tory vesicles are present and show the normal structures found in a sixty-eight-hour embryo. The various structures in the entire trunk region correspond to the normal condition and need no further description. The intestinal groove (*I.Gr.*) is unusually well developed and can readily be distinguished.

There are two distinct hearts present, (1) and (2), as indicated in the figure. Judging from the single well-developed vitelline vein of either heart, the two hearts began as two parallel endocardial tubes, which failed to unite into a common tube, and later either independent tube resulted in a distinctly normal heart. Either heart is normal in every respect. The embryo, as in figure 5, showed an alternate pulsation of the two independent hearts. The ventral aortae and aortic arches on either side are distinctly independent and do not show any crossing over. The aortic arches extend dorsally around the fore gut to the dorsal aortae, which unite into a single vessel posteriorly. The aortic arches in heart (1) are of the usual type, and extend dorsally through the gill arches. Two gill slits, corresponding to the first and second are present. The aortic arches of heart (2) differ from those in (1). No gill slits are present. The vitelline veins of either heart continue forward from the blastoderm in their corresponding splanchnic fold.

Either heart projects unusually far into the extra coelom. There is no indication that the hearts will later be drawn into the body cavity. This condition is partially due to the poorly developed somatopleuric folds in the head region.

The fore gut is abnormally wide and shows but two gill slits present on the right side. The hind gut and allantois are normal.

Columbia, Mo.

February 13, 1919

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Resumen por el autor, Leo Carl Massopust.
Universidad Marquette, Milwaukee.

Un método simple para preparar vidrios de luz diurna para
trabajos microscópicos.

El vidrio empleado por el autor es blanco, esmerilado en una de sus superficies; el color un tubo de "azul permanente" al óleo. Se coloca un poco de color en una vasija, se añaden unas cuantas gotas de trementina y se mezcla bien. Se toma un pedazo de gasa fina, y con él se envuelve un poco de algodón formando una muñequilla. Se impregna esta con el color y se frota ligeramente sobre la superficie esmerilada del vidrio teniendo cuidado de mantener el mismo tono. Cuando se ha aplicado el color la superficie del vidrio adquiere un aspecto granuloso o punteado, que produce la luz blanca. Por este método se obtiene una luz blanca brillante, aun más blanca que la que se obtiene usando los vidrios productores de luz diurna que se encuentran en el comercio.

Translation by José F. Nonidez
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A SIMPLE METHOD OF PREPARING DAYLIGHT GLASS

LEO C. MASSOPUST

Marquette University, School of Medicine

Owing to the almost universal use of daylight glass, a simple and inexpensive method of preparing daylight glass for microscopic work may prove of interest to the laboratory worker, particularly at the present time, when it is difficult to procure the commercial article.

The glass used is white glass ground on one side; this may be procured from any glass dealer and cut to the required size. For the color a tube of permanent blue oil color can be obtained at any artist's supply house. (We obtained the best results by using pigment manufactured by the Devoe & Reynolds Co.) Some of the pigment is placed in a small dish, a few drops of turpentine added, and mixed so that the consistency is that of a soft paste. A small piece of finely meshed gauze is taken, a small wad of cotton is placed on the gauze and gathered up at the ends so as to make a dauber (pounce). The dauber is dipped into the preparation described above, and dappled (not rubbed) on the ground surface of the glass. Care must be taken to maintain an even tone over the entire surface. When this even tone has been produced, the surface will have a stippled effect, which effect accounts for the white light. If this preparation is applied as a flat tone, no matter how light the tone, the field will be blue. Therefore, it is very necessary to have some of the yellow light come through with the blue. A microscope and microscope lamp may be kept in readiness while preparing the glass to ascertain whether the field is too blue or too yellow. Should there be too much color on the glass, it may be removed by pressing a clean portion of the dauber firmly on the glass and then lifting it off in a vertical direction without any rubbing whatever. If there is not sufficient color another light coat of

the preparation may be applied until the proper density is obtained. The color is then allowed to dry and harden thoroughly. We have obtained by this method a bright white light, even whiter than that obtained by the use of the commercial daylight glass.

The glass so prepared has been used in our laboratories for several months with very satisfactory results. Examination of this glass by means of the comparison spectroscope shows little difference between glass prepared in the laboratory according to this method and the daylight glass obtained from dealers.

Resumen por el autor, Harrison Randall Hunt.
Universidad de West Virginia.

Nacimiento de dos fetos de gato (*Felis domestica*) desigualmente desarrollados.

Próximamente veinte y cuatro horas después de respirar aire con cloro, una gata parió un feto aparentemente normal, de 14.1 mm. de longitud. Tres horas más tarde nació un segundo feto a término, vivo. La madre trató de comerse al feto más pequeño y a esta causa se debe probablemente el aspecto fragmentado y la destrucción de algunos de sus órganos, y su aspecto en los cortes no se debe a condiciones patológicas sino a la acción de los agentes mecánicos que siguieron al nacimiento. Una comparación entre el feto más pequeño y los fetos normales de gato de aproximadamente, la misma edad, demostró que aquel es realmente normal en el aspecto exterior. No hay pruebas suficientes sobre la muerte prematura del feto más pequeño en un estado poco avanzado del desarrollo, porque su forma exterior estaba bien conservada. Del mismo modo, las condiciones patológicas, tales como las que se suponen que siguen a la malnutrición, faltaban en dicho feto. Probablemente la explicación más satisfactoria de la diferencia de desarrollo de los dos fetos es que los óvulos que los originaron fueron fecundados con un intervalo de varias semanas. Pueden pertenecer al mismo periodo de ovulación, pero fueron fecundados por espermatozoides introducidos en diferentes coitos (superfecundación) o la ovulación, seguida de la fecundación, puede haber ocurrido durante la preñez (superfetación).

Translation by José F. Nonidez
Carnegie Institution of Washington

BIRTH OF TWO UNEQUALLY DEVELOPED CAT FETUSES (*FELIS DOMESTICA*)

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Department of Zoology, West Virginia University

TWO FIGURES

In April, 1918, while conducting some experiments with the domestic cat on the effects of inhaling chlorine gas, Dr. William H. Schultz, at that time head of the Department of Pharmacology of the Medical School of West Virginia University, observed an interesting case. He very kindly presented the material and data to the writer, who was assisting him in the investigation.

Shortly after 11 a.m. on April 6, 1918, a pregnant cat was placed in a closed container where it breathed chlorinated air for twelve minutes. It was then removed and put back in its cage, looking very sick. With Doctor Schultz's permission, the following is quoted from his note book.

Apr. 7, 1918. 12.00. At about this time I observed the cat cleaning the vulva and eating a small bladder. Close examination revealed a small embryo. The gassing had caused violent contraction of the uterus.

3 P.M. This cat delivered at least one full term kitten with hair, which I collected along with the placenta. Whether the cat ate other embryos or not I can not say. Etherized it and placed in Bouin's fluid.

The smaller fetus was fixed in the same way.

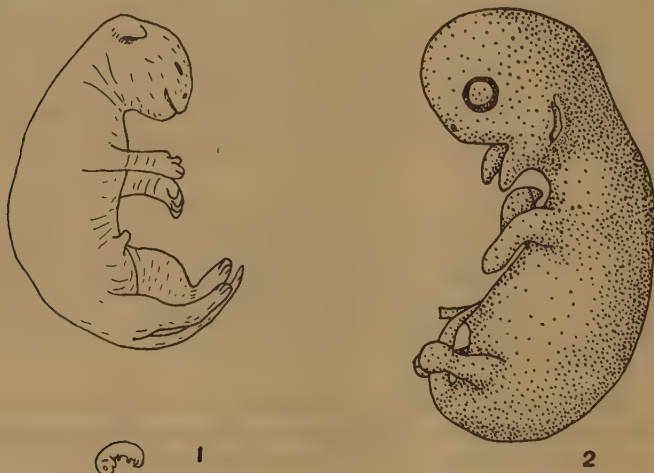
The facts certainly suggest that the inhalation of the chlorine had caused the cat to abort, though the larger fetus was so mature that it might have been born on the 7th in the absence of the chlorine treatment.

According to the writer's recollection, the fetal membranes of the smaller fetus were much torn, probably by the mother cat's

teeth. Unfortunately, they were not saved for histological study.

Figure 1 is an accurate outline of the fetuses made from a photograph taken by Dr. A. M. Reese. The outline shows accurately the relative sizes of the two. The crown-rump measurements were 105 mm. and 14.1 mm., so that the larger fetus was about seven and a half times as long as the smaller. The former was beyond doubt practically a full-term kitten.

Figure 2 shows the smaller fetus in greater detail. The mouth, eyes, tail, external ears, and limbs were well developed, and the olfactory pits were visible. The digits did not show externally.



The smaller fetus was compared with two normal cat fetuses, each 17.5 mm. long, the only ones of comparable age that could be secured. In these normal fetuses the digits were plainly visible, the nasal region had begun to protrude, and the eye and external ear were somewhat more developed than in the aborted fetus. Otherwise, the normal ones resembled the aborted fetus rather closely, except that the latter's mouth was open, showing the tongue, and the chin did not touch the breast as in the normal fetuses. Probably the mother's attempt to eat the fetus pulled the head backward and forced the mouth open. In external appearance this fetus certainly appeared to be normal.

It was impossible to determine accurately the age of the smaller fetus, for illustrations of cat embryos of different ages were not available. When compared with His' figures of normal human embryos (Keibel and Mall, vol. 1, p. 61), the smaller fetus was seen to correspond approximately, judging from its external appearance, with a forty-day human fetus. Such a fetus has passed through about one-seventh of its period of development. Since the period of gestation of the cat is about eight weeks, it would seem (though this estimate is necessarily a rough one) that the smaller cat fetus was in the second week of development.

A study of serial sections of the smaller fetus showed the following conditions. The cerebral hemispheres and body cavity had collapsed. The linings of the hemispheres and stomach were badly fragmented. In the lumbar and sacral regions a longitudinal ragged crack on the dorsal side of the body extended down into the spinal cord, disorganizing it extensively. In the neck region the cord appeared to have been slightly twisted on its long axis. The auricular walls of the heart were much cracked and fragmented. Many of the kidney tubules were normal. The walls of other tubules were more or less broken up. The outlines of the organs could be clearly made out. There was evidence of nuclear fragmentation, especially near the surface of the body, but for the most part the nuclei were normal in appearance.

Considering that the mother attempted to eat the fetus, it is not surprising that its delicate tissue showed mechanical injury. Probably the mother seized the ovum in such a way that pressure was brought to bear on the sides of the body, causing the coelom, auricles, cerebral hemispheres, and stomach to collapse. The resulting impact of the opposite walls of these cavities probably fragmented the walls as described above. The time which elapsed between the birth of the fetus and its immersion in the fixing fluid is not known. Possibly the nuclear fragmentation and the disintegration of some of the kidney tubules were due to postmortem autolytic processes following its expulsion from the uterus.

The difference in the degree of development of the two fetuses must have been due to one of the four following causes:

1. Both ova may, conceivably, have been fertilized at about the same time, one dying and remaining in the uterus for five or six weeks while the other continued its normal development. Dead human fetuses have been known to remain for days in the uterus without undergoing extensive changes (De Lee, '15). But frequently they undergo liquefaction, maceration, saponification, mummification, putrefaction, etc. (De Lee, '15; American Text-book of Obstetrics, '96; Edgar, '03). Stockard and Papanicolaou ('18) have observed degenerative changes in guinea-pig embryos. It may not be possible to demonstrate beyond all doubt that the difference between the two fetuses was due to some cause other than the death of the smaller fetus. But it seems probable that a dead fetus would have shown some gross external evidence of dissolution within five or six weeks after death. This fetus certainly showed no such changes.

2. Another possibility is that pathological processes appeared in one of the fetuses during the first week or so, retarding its development. Of the one hundred pathological human embryos studied by Mall ('08), representing stages of development from two weeks on, only 8 per cent, or one-twelfth, had a normal external appearance. If pathological processes retarded the development of the smaller cat fetus, they must have appeared early, possibly in the first or second week. Eleven out of twelve of Mall's embryos were abnormal in external appearance. Therefore, it is highly improbable that pathological changes extensive enough to retard development so decidedly in the smaller fetus could have acted for several weeks without causing gross external abnormalities.

3. It is conceivable that the smaller fetus developed more slowly than its litter mate as a result of malnutrition. Unfortunately, the blood supply of the uterus was not available for study. However, the observations of Mall show that malnutrition is one of the chief causes of pathological changes in the developing ovum. "It is no longer necessary," says Mall, "for us to seek for mechanical obstructions which may compress the

umbilical cord, such as amniotic bands, for it is now clear that the impairment of nutrition which naturally follows faulty implantation, or the various poisons which may be in a diseased uterus, can do the whole mischief" (Mall, '10, p. 240). Since no pathological changes were observed in this fetus, the hypothesis of malnutrition is certainly not supported by the facts.

4. Probably the most satisfactory explanation is that the eggs from which the two fetuses developed were fertilized several weeks apart. They may have belonged to the same period of ovulation, but were fertilized by spermatozoa introduced by separate coitions (superfecundation); or ovulation, followed by fertilization, may have occurred during pregnancy (superfetation). In the following discussion these definitions of the terms are used.

Cristopher ('86) believes that the cat can ovulate during pregnancy. Jepson ('83) reports the case of a cat which gave birth to an immature fetus in the same litter with two full-term kittens—possibly an instance of superfetation. Harman ('17) has reported a cat whose uterus contained three fetuses which "were developed near to term, and one apparently was much smaller, and showed a much less degree of development." She applies the term superfetation to this condition. Harman ('18) describes also a probable case of superfetation in the cow.

King ('13) concludes that superfetation and superfecundation occasionally occur in the albino rat. Both of these phenomena have been observed in man (Scott, '17).

Sumner ('16) believes that mouse eggs ovulated during pregnancy may be fertilized, one period of gestation being thereby imposed upon another.

The writer is unable to decide between superfetation and superfecundation as the true explanation of this case, though probably one or the other is the real explanation. If it be the latter, the two ova belonged to the same period of ovulation, but one was soon fertilized, while the other was fertilized several weeks after ovulation. Whether cat ova can retain their vitality this long is an open question, though Sumner's observations ('16) led him to believe that the spermatozoa of mice may retain their fertilizing power for days or weeks.

In the cat reported by Harman ('17) one of the more advanced fetuses lay between the smaller fetus and the ovary. A normally implanted ovum like the former might offer an impassable barrier to an egg ovulated during pregnancy. She suggests, therefore, that all the ova belonged to the same period of ovulation, but that one of them was fertilized later than the others.

In the case under discussion, however, the observed facts do not rule out the possibility of superfetation. Possibly the older fetus occupied but one horn of the uterus, the spermatozoa thereby having access to eggs ovulated during pregnancy. Longley ('10, '11) observed in the cat that ovulation and maturation of the egg depend upon copulation. This suggests that coition during pregnancy may have led to the liberation (Cristopher, '86) and fertilization of the ovum from which the smaller fetus developed.

The probabilities certainly are that the case described is either one of superfecundation, in which the fertilization of the egg was delayed for weeks, or a case of superfetation.

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Resumen por el autor, Edgar F. Cyriax.
Londres.

Nota sobre la clavícula "flotante."

El nombre de clavícula "flotante" se aplica a una estructura no rara, aparentemente adquirida, en la cual el extremo interno de la clavícula en vez de reposar sobre la faceta articular del esternón formando con ella una articulación, está completamente libre y es móvil. La amplitud de movimiento en diversas direcciones es, en general, próximamente de un cuarto de pulgada. Esta estructura se ha observado particularmente en el lado izquierdo. No se descubrieron síntomas subjetivos.

Translation by José F. Nonidez
Carnegie Institution of Washington

A BRIEF NOTE ON "FLOATING" CLAVICLE

EDGAR F. CYRIAX

London

I have ventured to apply the name of "floating" clavicle to a condition which as far as I know has up to the present not been described, namely, one in which the inner end of the clavicle instead of constantly impinging upon and forming a joint with the articular facet on the sternum, lies quite free and moveable.

I am inclined to think that the condition is not so uncommon, and that the reason why it passes unnoticed is simply because it is not looked for, inasmuch as it apparently produces no subjective symptoms; in the cases I have seen the patient himself was not even aware of the abnormality until it was pointed out to him. My attention was first drawn to the condition of floating clavicle about twelve months ago, when I discovered it while examining the joints in a case of articular rheumatism of the left arm; the left sterno-clavicular joint was the one that was involved. Six months later I found another floating clavicle, also on the left side, in a girl of ten who stammered, and recently I found a third case again also on the left side, in an aviator who informed me that he had once had phthisis pulmonum some years previously.

In none of these cases were there any subjective symptoms whatever. They all showed practically the same objective ones, as follows: The inner end of the clavicle was free and was not in contact with the sternum; when the arm was dependent it occupied a position about one quarter of an inch above its fellow on the opposite side. On grasping the sternal end of the clavicle between the forefinger and thumb it was found that it could perfectly easily be moved downwards onto the articular facet on the sternum, and from there with equal facility upwards, inwards, outwards, backwards and forwards. The range of all

these movements was about the same, namely approximately one quarter inch; the final stage of each movement was somewhat abruptly limited, suggesting that this was done by means of ligaments rather than muscles. The only one of these movements that had any result was the backwards one which caused some irritation of the larynx; none of the others caused even a passing inconvenience, although the inwards movement seemed to exercise a fair amount of pressure on the sterno-mastoid, causing it to bulge locally.

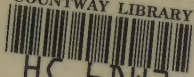
On moving the clavicle away from the sternum, palpation of both articular surfaces could be done with fair accuracy; as far as could be judged both were normal as regards surface and outline. This leads me to suppose that the condition is acquired rather than congenital.

In none of the subjects examined did the condition of the clavicle seem to have any effect on the strength of the muscles or the range of the movements of the shoulder girdle. On endeavouring to replace the clavicle into its joint, this as stated above could readily be done, but displacement occurred as soon as the patient attempted movements which threw any strain on the joint, even when these movements were executed only through a very small range. In two of the cases mentioned medico-gymnastic treatment was applied in order to try and effect a permanent reposition of the joint, but in both cases no improvement at all resulted.

In none of the three cases was I able to find any evidence that the floating clavicle was responsible for any of the symptoms, excepting perhaps in the patient who stammered. Her chief impediment was spasmodic action of the diaphragm, and it is not impossible that the free movements of the sternal end of the clavicle may have acted as a continued source of irritation to the phrenic nerve. In her case however pressure of the bone backwards did not aggravate the condition.

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